THE BIOLOGICAL ELIMINATION OF PHENOLS IN THE EFFLUENT OF A WOOD PRESERVING PLANT

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The removal of phenols from the wastewaters of wood preserving plants has always presented problems. The purpose of this paper is to investigate the possibility of employing a biological system to reduce the phenol content of effluent from these plants. The data concerning this possibility were obtained through basic research and are found in the section entitled Results.

This thesis contains sections entitled Introduction, Materials and Methods, Results, Discussion, Conclusion, and Literature Cited. The Introduction describes past research involving phenol removal. The Materials and Methods section gives details of the research procedures employed in this investigation. The Results section contains the findings of the research and is followed by the Discussion which evaluates those findings. Obvious implications of the research are presented in the Conclusion. A list of references is given in the Literature Cited section.

From the research conducted it was confirmed that rapid phenol removal may be obtained in the plant effluent following the addition of ammonium phosphate and subsequent aeration. It was also discovered that sudden exposure of the system to

high concentrations of phenol results in a lag phase prior to rapid phenol removal. This lag phase may be shortened or removed following the conditioning of the bacteria to gradual increases in the phenol concentration. The bacteria in the effluent of the wood preserving plant investigated were found capable of rapid phenol removal and the introduction of additional phenol degrading bacteria was not necessary.

It was concluded that a biological system for phenol removal is possible in treating wood preserving plant wastewaters, if the nutrient deficiency of the effluent is recognized and supplemented and adequate aeration is supplied.

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THESIS

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By

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

INTRODUCTION

Pollution of waters by phenols presents problems of taste, odor, and toxicity. Concentrations as low as 0.001 parts per million (ppm) will produce objectionable taste and odor in chlorinated waters, and concentrations as low as 0.01 ppm present similar problems in nonchlorinated waters (32). Toxic amounts of phenols render the water useless to man and destroy its normal life-sustaining functions.

Phenols occurring naturally in water and soil are most often found in low concentrations. Degradation by bacteria and fungi will remove these low concentrations; but high concentrations, such as those found in the wood preserving industry, present a more formidable task. Both chemical and biological methods have been tested and proven effective in phenol removal from industrial effluents.

Most chemical methods of phenol removal depend on the utilization of strong oxidizing agents capable of oxidizing phenols to non-toxic intermediates. Eisenhauer (10) suggested hydrogen peroxide and a ferrous salt reagent (Fenton's Reagent) as a phenol oxidizing additive. This oxidation process yielded catechol and hydroquinone as intermediates, which were then oxidized to the corresponding quinones. Hall and Nellist (15) suggested ozone as an oxidizing agent capable of phenol removal. They found that when ozone was introduced into the

effluent from a coke plant it could cause the reduction of 2000 ppm of phenol to 1 ppm. Eisenhauer (11) found catechol (dihydroxy benzene) as the primary intermediate in ozonization, followed by o-quinone and p-quinone. Clevage of the quinones yielded cis, cis-muconic acid, which eventually oxidized to maleic, fumaric, or oxalic acid. Spicher and Skrinde (28) suggested potassium permanganate as an oxidizing agent and implicated dihydroxy benzene as the initial intermediate followed by quinone.

Biological elimination of toxic amounts of phenols has proven in the past to be a reliable and economical method of phenol removal. Putilina (23) investigated phenol removal in coke plant effluent. He inoculated an "aerotank" with bacteria capable of using phenols at a rate of 200 ppm in 24 hours at a pH of 7.6 to 7.8 and temperatures ranging from 25 to 30° C. Degradation was also possible when the concentration was 1000 to 2000 ppm phenol. Smith (27) described several methods of biological degradation of phenols in chemical plants owned by Dow Chemical Company. The first of these plants was built in the mid-1930's and consisted of a primary clarifier accepting diluted effluent containing approximately 75 ppm phenol. This effluent passed through a trickling filter and finally went to a series of oxidation ponds. In the present installation, an activated sludge section has replaced the ponds. The second plant, constructed in 1958, consisted of two separators, a system of trickling filters;

and an activated sludge section followed by a clarifier. In 1961 Dow constructed a third plant, with waste disposal systems similar to the previous one but with a major alteration. An equalization pond prior to the filtration tower was added. These waste disposal units were capable of removing 99 per cent of the phenols in the Dow chemical plant effluents. Biczysko and Suschka (8) investigated phenol removal in oxidation ditches. These ditches were approximately 200 cubic meters in volume and were equipped with two aeration rotors. The ditches were designed to treat 80 to 120 cubic meters of effluent per day at concentrations of 500 to 1200 ppm phenol. They found almost total removal of phenolic compounds. Nakashio (22) described phenol removal in coal gas washing disposal waste using an activated sludge process. This method was used at the Hiroshima City Gas Company. Flocs of activated sludge prepared from horse and cow dung were capable of removing 99 per cent of the phenol present. The system operated well at concentrations as high as 1200 ppm. Prasad et al.(24) investigated the use of high-rate deep trickling filters for phenol removal in a wood carbonization plant. When concentrations were no greater than 100 ppm, they obtained 90 per cent removal of phenols in a period of time suitable for plant operations.

Dagley and Patel (6) conducted extensive studies into the oxidation of p-cresol and related compounds by a bacterium they isolated from liquids that had circulated through a

coal gas and fuel production plant. They identified this organism as a species of Pseudomonas. Seenson and Walker (30) discussed the decomposition of 2,4-dichlorophenol by a strain of Achromobacter isolated on media containing 2,4-dichloro phenoxyacetic acid. Davey and Turner (5) examined three phenol-decomposing strains of bacteria they identified as Pseudomonas. Chambers, Tabal, and Kabler (3) investigated the degradation of 48 different phenolic compounds by mixed cultures of bacteria they had obtained from soil, compost, or mud from the waste lagoon of a catalytic cracking plant. These mixed cultures were predominantly pseudomonads. Ingols and Stevenson (16) studied the biodegradation of phenolic pesticides containing carbon to chlorine bonds. They utilized an activated sludge from the Dow Chemical Company but found it lacked activity in the degradation of chlorinated phenols. Limited decomposition of eight of the nine chlorinated phenols tested was obtained. Ribbons (25) studied the kinetics of oxidation of several phenols by Pseudomonas aeruginosa. He suggested 3-methylcatechol as the primary intermediate in o-cresol degradation and catechol as the primary intermediate of phenol degradations.

Jayasankar and Bhat (17) isolated strains of Micrococcus and Pseudomonas from the rets of coconut husk which were capable of oxidizing phenolic substances. They found, as had Ribbons (25), that catechol was the primary intermediate of phenol oxidation. Bayly, Dagley, and Gibson (1) utilized

Pseudomonas to degrade phenol to its intermediate catechol and o-cresol to its intermediate 3-methylcatechol.

In spite of the work cited above, the reduction of phenols in wastewaters from wood preserving plants has always presented a problem. Work has been limited primarily to companies in this field or to researchers employed by these companies. A special subcommittee of the Information and Technical Development Committee (7) of the American Wood-Preserver's Association (AWPA) reported in 1956 and 1957 on the problems confronting the industry but had few suggestions for the removal of phenols. In 1958 a special Wastewater Disposal Committee report (18) suggested phenol removal by deep well injections, evaporation, incineration, solvent extraction, chemical oxidation, and biological oxidation. The biological method was suggested as one of the most economical of the aforementioned methods. Halff (14) in 1959 suggested slow sand filtration as a method for treatment of plant effluents. He measured the efficiency of the treatment process by determining the reduction in toxicity of the effluent to fish. The 1960 Wastewater Disposal Committee (19) of the AWPA suggested removal of phenols by an activated sludge system. This system was capable of reducing phenols from 2000 to 1 ppm. Gaudy, Scrudder, Neeley, Perot, and Crane (13) investigated spray irrigation and oxidation ponds as a method of improving wood preserving plant effluent. The spray irrigation method reduced the phenols

by about 50 per cent while the oxidation ponds removed approximately 94 per cent of the phenols in six days. In 1969, von Frank and Eck (33) suggested a chemical method of treatment. They found that the addition of caustic soda (sodium hydroxide) and an aqueous suspension of lime (calcium hydroxide) reduced the phenols by about 50 per cent. The Wastewater Disposal Committee (ITD-2) of the AWPA reported in 1970 (26) that the Southern Pressure Treaters Association had entered into a contract with Mississippi State University. Under the terms of the contract, Mississippi State University would carry out a general study of ways and means for reducing pollution and the handling of wastewaters at wood preserving plants. The work is yet unfinished but early reports suggest a combination of chemical and biological treatments.

The following study presents an investigation of the phenol-degrading bacteria which are capable of reducing the phenol concentrations in wood treatment plant effluent. It also investigates methods of enhancement of phenol reductions as well as rates of degradation.

MATERIALS AND METHODS

Collection of Samples for Biological Oxygen Demand, Chemical Oxygen Demand, and Phenols

Samples were collected from the plant effluent of the W. J. Smith Wood Preserving Plant at Denison, Texas. Three samples were collected during alternating days of each week from July 23, 1970, until the time at which this paper was prepared. The normal times of collection were 8:00 a.m., 12:00 noon, and 4:00 p.m. Samples were collected in one-liter Nalgene bottles and kept in ice until chemical analyses were conducted in Denton, Texas. A composite sample was prepared by combining varying amounts of each of the three samples. The amount of a particular sample added in preparing the composite depended on the flow rate at the time of its collection relative to the flow rate of the other two samples. In this manner, the sample reflected daily output of effluent from the plant.

Phenol Determination (Standard)

The aminoantipyrine method described in the Twelfth Edition of Standard Methods for the Examination of Water and Wastewater (29) was employed for the phenol determinations. This particular method employs a preliminary distillation to reduce optical turbidity and color and to rid the sample of possible interfering chemicals. Following the distillation, a suitable

amount of the sample, dependent on the phenol concentrations, was diluted to 100 ml, 2.0 ml of ammonium chloride was added, the pH adjusted to 10 $\pm$ 0.2, and then 2.0 ml of potassium ferricyanide solution and 2.0 ml of aminoantipyrine solution were added. The optical density of the resulting color was measured at 510 millimicrons (mm) on a Bausch and Lomb Spectronic 20, and compared to a reference curve.

Phenol Determination (Modified)

A modification of the above method proved to be less time-consuming but accurate enough to justify its use. In samples with extremely high phenol concentrations, such as 200 ppm or above, and where dilution of the sample reduced the optical turbidity or color to the point that it did not markedly interfere with the optical density reading, the modified method proved advantageous. This method simply bypassed the distillation step and depended on centrifugation of the sample at 10,000 revolutions per minute for ten minutes to reduce the optical turbidity. Following the substitution of centrifugation for distillation, the procedure was the same as the standard method. Upon comparison, the modified aminoantipyrine method was found accurate when the aforementioned qualifications were met.

Chemical Oxygen Demand (COD)

COD determinations were conducted in accordance with the Twelfth Edition of Standard Methods (29), utilizing the

dichromate reflux method. A COD determination was conducted weekly on the wastewater samples.

Biochemical Oxygen Demand (BOD)

BOD determinations were conducted in accordance with the Twelfth Edition of Standard Methods (29), employing the azide modification of the iodometric method. Isolated bacteria capable of utilizing creosote as a sole carbon source were used as seed. These isolates were inoculated into a 500 ml flask containing 300 ml of Tryptic Soy Broth, and incubated at room temperature for 24 hours on a reciprocal shaker. Fifty milliliters of this 24 hour culture were centrifuged and washed two times with distilled water in a Sorvall Superspeed RC2-B Automatic Refrigerated Centrifuge. The washed bacteria were then resuspended in 500 ml of distilled water. Twenty milliliters of this cell suspension were added to a BOD bottle along with 280 ml of "BOD water" to determine the BOD of the seed. One milliliter of seed suspension was added as inoculum to the BOD bottles, which contained the wastewater sample plus the BOD water. The BOD determination followed a five-day incubation period in a Scientific Systems Environmental Chamber at 20^{o} C.

Bacterial Counts

The serial dilution and spread plate technique was employed to determine the number of viable bacterial cells in all effluent samples tested. Tryptic Soy Agar constituted

the plate count media. The plates were incubated at 37° C for 48 hours prior to counting.

Collection of Soil and Water Samples for the Isolation of Phenol Degrading Bacteria

Fifteen soil samples were collected from the premises of the W. J. Smith Wood Preserving Company. At the same time three water samples were collected from a stream contaminated with the plant effluent. Isolations were also made from 25 soil samples collected from the bases of telephone poles in Denton, Texas. Soil and water samples were collected in air-tight cellophane bags and processed as soon as possible.

Composition of Media Employed to Isolate Creosote Utilizing Bacteria

A modification of L-Salts medium (21) was employed to isolate the bacteria. Its composition is as follows:

Na_2HPO_4	0.21	grams
NH_4NO_3	2.0	
$MgSO_4 \cdot 7H_2O$.	0.2	
$FeSO_4 \cdot 7H_2O$.	0.001	
K_2HPO_4.	0.21	
KH_2PO_4.	0.09	
$CaSO_4 \cdot 5H_2O$.	5	milligrams
H_3BO_3	10	
$MnSO_4 \cdot 5H_2O$.	10	
$ZnSO_4 \cdot 7H_2O$.	70	
MoO_3.	10	
KCl	40	
$CaCl_2$	15	

Isolation of Bacteria Capable of Utilizing Creosote as a Sole Carbon Source

One gram of soil sample or one milliliter of water sample was added to 100 ml of basal salts medium in 250 ml Erlenmeyer flasks. Creosote at a concentration of one tenth of one per cent represented the carbon source. These flasks were then placed on a reciprocal shaker at room temperature for four days. At the end of the fourth day, one milliliter of those samples showing the greatest turbidity was transferred to another 250 ml Erlenmeyer flask with sterile basal salts medium and creosote. These cultures were also incubated for four days. From these cultures were obtained isolates by streaking portions of the growth onto Tryptic Soy Agar plates and plates of the basal medium containing creosote to final concentrations of 0.1 to 0.3 per cent. The plates were incubated for 48 hours at 37° C. Isolated colonies were then transferred to semi-solid agar, incubated 24 hours at 37° C and then refrigerated.

Anaerobic and Aerobic Degradation of Phenols

To determine the possibility of utilizing an anaerobic system for phenol reduction in creosoting plant effluent, an experiment was designed which compared the phenol reduction rate of an anaerobic system with that of an aerobic system. The inoculum for both systems was prepared by inoculating 500 ml of Tryptic Soy Broth with different isolates. Fifty milliliters of the inoculum were added to a five-gallon

Nalgene container. The container was then filled with plant effluent and sealed to exclude air. This provided an adequate anaerobic system. The aerobic system was prepared by adding two and one-half gallons of effluent to a five-gallon Nalgene container. The system was aerated by passing air through a filter and then bubbling it through the effluent. The samples were incubated at room temperature. The phenol content of the anaerobic and aerobic systems was determined by the aminoantipyrine method, and the results compared as shown below.

Confirmation of Biological Degradation of Phenols

This experiment was conducted to examine the possibility of reduction in phenol concentration by physical or chemical reactions in the culture systems under study. To two 2000 ml flasks were added 1000 ml of plant effluent and washed bacteria. Both flasks were exposed to aeration. One gram of mercuric sulfate was added to one flask in order to stop or at least reduce biological activity. Bacterial counts were made on each flask, employing the serial dilution and spread plate technique. Counts were made on Tryptic Soy Agar plates after incubation at 37° C for 48 hours. The bacterial populations of the cultures containing mercuric sulfate were reduced to less than 10^2 organisms per milliliter and it was assumed that these were resting cells or spores. Thus, by determining the reduction rate of phenol in the two flasks, a

comparison could be made between the biological and the chemical or physical effect of the system.

Variations in Tryptic Soy Broth Concentrations

It was found that Tryptic Soy Broth acted as a growth enhancer in the effluent cultures. A series of experiments was conducted to determine the effect of variations in the Tryptic Soy Broth concentration on the phenol-reduction rate. To four 2000 ml Erlenmeyer flasks were added 1000 ml of plant effluent, 800 ppm phenol, and varying concentrations of Tryptic Soy Broth. These were inoculated with different bacterial isolates. The flasks were aerated by forcing air through the liquid. Phenol determinations were made over a 72-hour period, employing the modified aminoantipyrine method.

Ammonium Phosphate, a Supplement for Tryptic Soy Broth

It was deemed essential to find an economical method for the reduction of phenols in industrial effluents. This required substitution of an economical growth supplement for Tryptic Soy Broth. Ammonium phosphate, an inexpensive nitrogen and phosphorus source, was studied in the culture systems under investigation. This experiment was conducted to determine the growth-enhancing properties of ammonium phosphate as compared with Tryptic Soy Broth. To five 2000 ml flasks was added plant effluent containing approximately 200 ppm phenol. The first flask contained no Tryptic Soy Broth or ammonium phosphate and served as a reference. Flasks

numbered 2 and 3 contained 1 and 5 per cent Tryptic Soy Broth, respectively; flasks numbered 4 and 5 contained 1 and 5 per cent ammonium phosphate, respectively. Washed bacteria were added to all the cultures. The flasks were aerated and phenol determinations conducted over a 72-hour period employing the modified aminoantipyrine method.

Development of Inoculum Capable of Rapid Phenol Degradation

The purpose of this experiment was to develop an industrial inoculum capable of removing high concentrations of phenol in wastewater at a rapid rate. It also served to assess the possibility of enhancing the rate of phenol removal by inoculating with the creosote-utilizing isolates. One thousand milliliters of plant effluent and 1000 ppm ammonium phosphate were added to two 2000 ml flasks. Flask number two was inoculated with different bacterial isolates and flask labeled number one served for reference. Both flasks were aerated by shaking on a reciprocal shaker at 25° C for 24 hours. At the end of this 24-hour period, 400 ppm phenol were added to a set of two flasks. Phenol determinations were performed at 0 and 24 hours. After the 24-hour determinations, 400 ppm phenol were again added to both flasks and phenol determinations repeated at that time and also twelve hours later. At this point 500 ppm phenol were added to each flask and the phenol concentrations determined then, and also after four and eight hours. The flasks were

then exposed to three separate additions of 1000 ppm phenol over a 48-hour period. Following the third addition, the phenol concentration was determined at zero, three and one-half, nine, and twelve hours.

RESULTS

The results obtained from the research described here can be divided into two major classes. The first experiments performed dealt primarily with a measurement of the extent of contamination of the plant effluent, with the bacterial population of the wastewater, and with the effect, if any, of temperature on these parameters. The second part of the research was designed to show the capability of certain bacteria to degrade phenol in laboratory-scale experiments and to investigate methods of enhancing the phenol degradation rate.

Part I

The first series of experiments gave results which were to a certain extent surprising. Although the plant wastewater was very obviously contaminated with creosote, oil, and phenolic substances, it harbored large populations of bacteria. The data given in Table I show the extent of the heterotrophic, aerobic, mesophilic organisms capable of forming macrocolonies on Tryptic Soy Agar after 48 hours incubation. Probably the actual number of bacteria and other microorganisms which normally inhabit the wastewater is larger by several orders of magnitude. Since the lowest plate counts obtained were 1.6×10^4, it can be stated that the wastewater in the creosoting plant contains a sizable microflora which

TABLE I

Bacterial populations in wastewater from the creosoting plant. Three samples were collected during the course of a day in each week of each month. The samples were combined to reflect flow rates in the final mixture.

Month	Bacteria per ml			
	1st Week	2nd Week	3rd Week	4th Week
July	*	*	9.9×10^6	2.0×10^6
August	3.2×10^6	4.9×10^6	1.2×10^6	---
September	4.6×10^6	1.7×10^6	2.3×10^6	2.0×10^6
October	5.3×10^6	4.6×10^6	1.7×10^7	8.4×10^5
November	3.9×10^5	2.1×10^5	1.0×10^6	---
December	5.9×10^6	4.9×10^6	1.8×10^5	1.6×10^4
January	2.0×10^4	1.8×10^6	4.0×10^5	1.6×10^4
February	1.0×10^6	1.0×10^6	6.8×10^6	6.8×10^6
March	6.7×10^5	4.1×10^4	3.1×10^4	---
April	3.3×10^6	3.8×10^6	3.4×10^6	4.8×10^6
May	6.4×10^6	6.6×10^6	4.8×10^5	---
June	8.7×10^5	4.0×10^5	5.4×10^5	---

*Experiment did not begin until the third week in July of 1970.

is indigenous to it and which undoubtedly represents those bacteria which normally exist in water containing phenolic substances. It is also probable that these bacteria bring about degradation of these materials in nature.

The fact that these bacteria utilize some of the organic constituents, such as phenol and closely related substances, as growth substrate is confirmed by the fact that they can grow in laboratory media where the wastewater is employed as one of the essential nutrients. The data in Table II show six bacteria isolated from soil wetted by the plant wastewater which are capable of forming macrocolonies in 48 hours in media containing 0.1 per cent creosote as the sole source of carbon. The fact that these bacteria are found in other locations is also illustrated by the data in this table.

Although the wastewater from the creosoting plant contains large populations of bacteria capable of using it as growth substrate, the equilibrium between bacteria and usable substrate is unknown. That is, do the bacteria present utilize all the organic constituents of the wastewater? Figure 1 shows the content of phenol in wastewater, Figure 2 the chemical oxygen demand, and Figure 3 the biological oxygen demand of this effluent. The data in Figure 1 show that phenol is always present in the wastewater regardless of the extent of bacterial populations. Figure 2 and 3 simply serve as indices of the total load of organic contaminants in the water. They are presented here because they bear out nicely the hypothesis

TABLE II

Bacterial isolates used in laboratory studies. A basal salts medium similar to L-Salts with creosote, phenol or glucose and pentachlorophenol as the carbon source was employed.

Isolate Number	Source	Carbon Source
1	Creek bank, Denison	0.1% creosote
2	Creek bank, Denison	0.1% creosote
3	Creek bank, Denison	0.1% creosote
4	Creek bank, Denison	0.1% creosote
5	Creek bank, Denison	0.1% creosote
6	Creek bank, Denison	0.1% creosote
7	Telephone pole, Denton	0.1% creosote
8	Plant effluent, Denton	0.1% creosote
9	Telephone pole, Denton	100 ppm pentachlorophenol
10	Telephone pole, Denton	100 ppm pentachlorophenol
11	Dust, Denton	200 ppm phenol
12	Phenol contaminated artesian well, Denison	200 ppm phenol

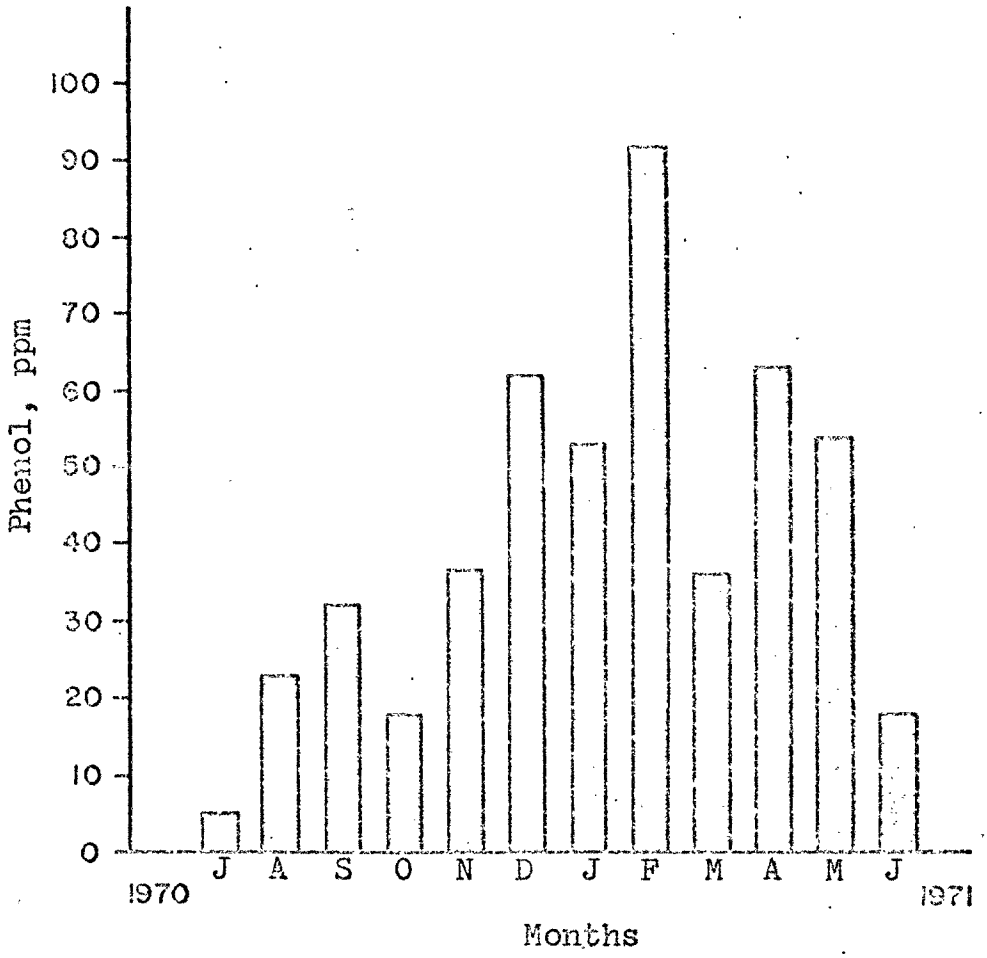

Fig. 1--Concentration of phenol in plant effluent; each bar represents the average of the weekly concentrations measured within each month.

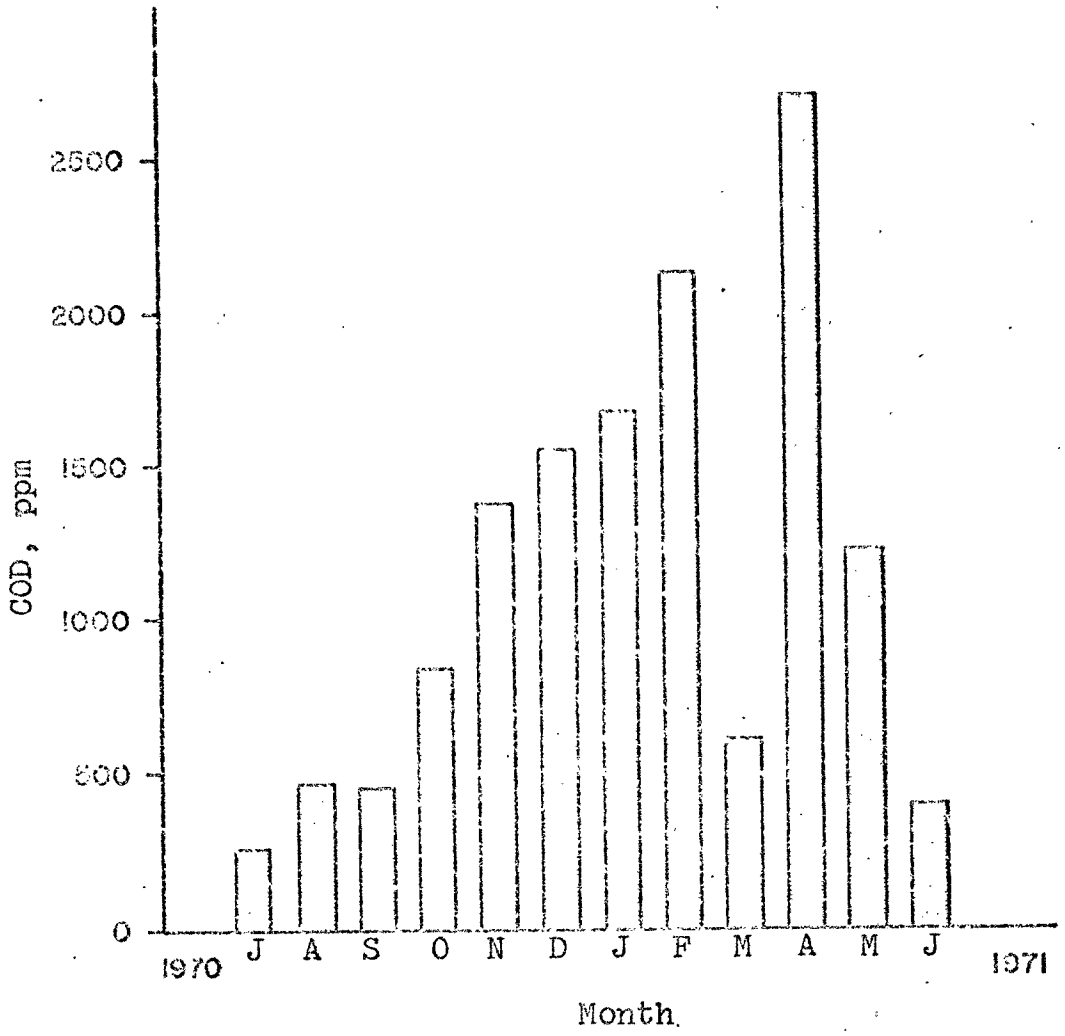

Fig. 2--COD of plant effluent: each bar represents the average of the weekly COD values for each month.

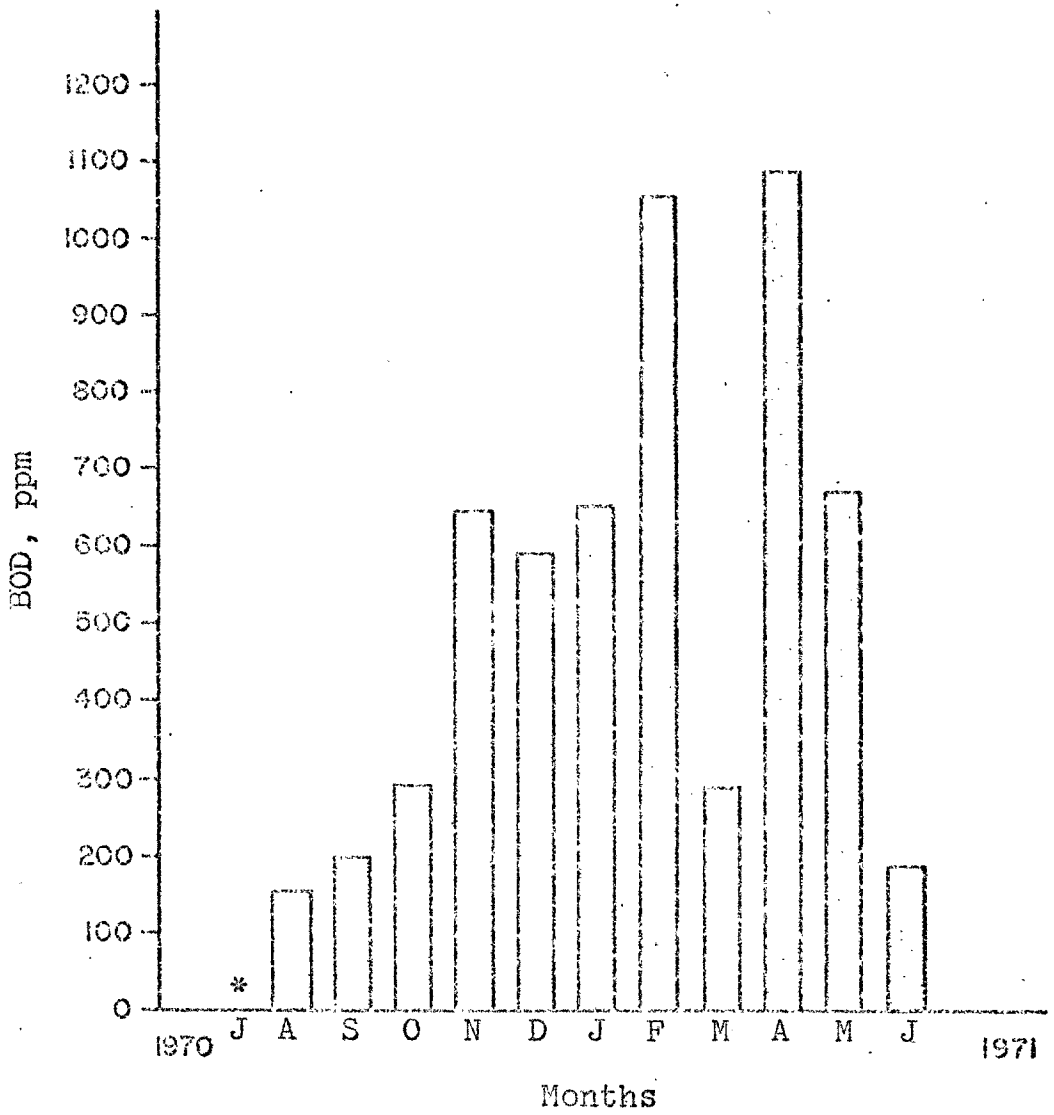

Fig. 3--BOD of plant effluent: each bar represents the average of the weekly BOD values for each month.

*Results not obtained

that wastewater purification in this system is, to a given point, a function of water temperature; see Figure 4. Comparison of Figures 1, 2, 3, and 4 will show this.

Part II

It had been determined that the plant effluent was constantly contaminated with phenol and that certain bacterial isolates were capable of phenol utilization. If the number of phenol-utilizing isolates could be increased, the phenol concentration might decrease. The inefficiency of the natural system was confirmed because of the constant presence of phenol in the wastewater. Under the assumption that the bacteria present failed to exert a hardfelt effect on the phenol concentration, due to their limited numbers, an experiment was designed to determine if an aerobic or anaerobic system would best support the phenol degrading population. Table III established unequivocally the fact that phenol removal is best obtained in an aerobic environment and that an anaerobic culture has little or no effect on the phenol concentration. The aeration of plant effluent was found to stimulate the growth of the bacterial population and concurrently the removal of phenol. It was at first assumed that the stimulation of the phenol removal rate was either biological in nature, stemming from the increased bacterial number, or abiological, resulting from the introduction of air into the effluent. Figure 5 indicates that following the

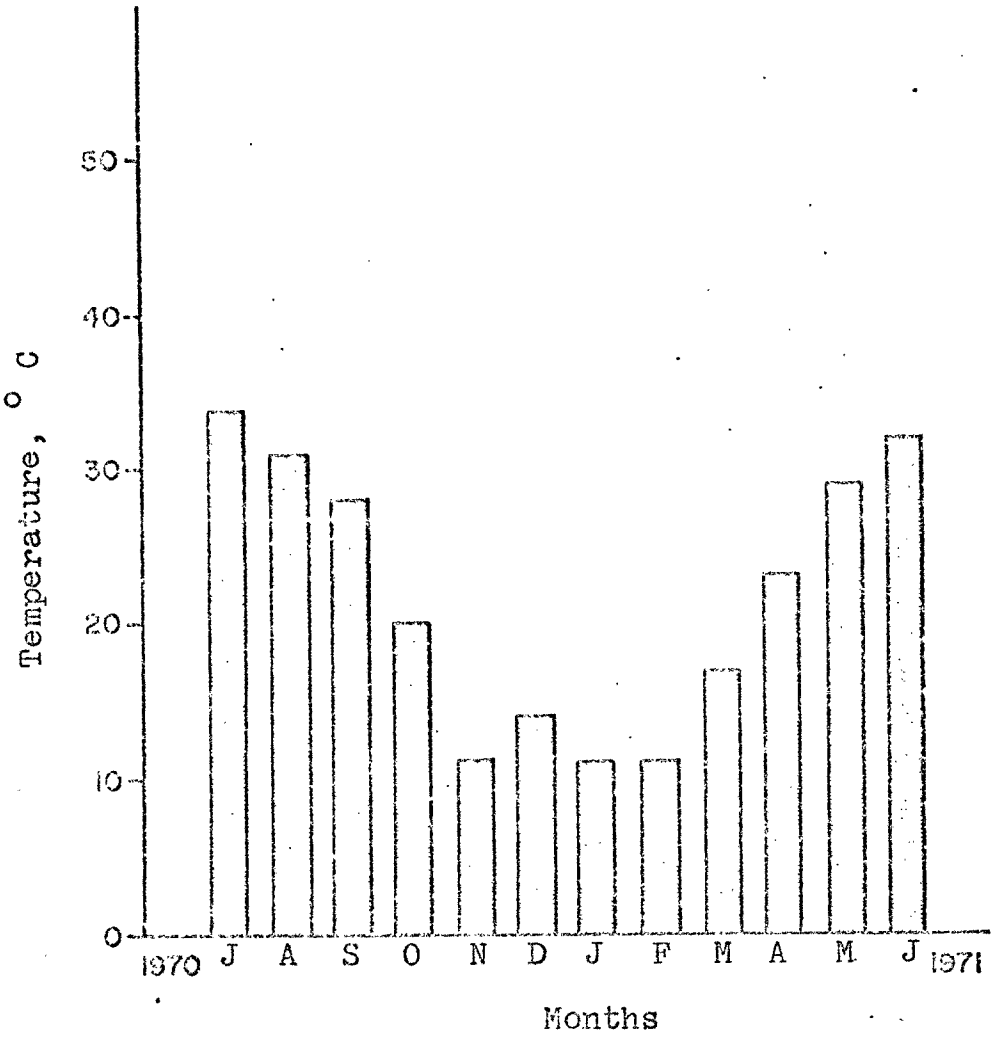

Fig. 4--The average of the weekly temperatures for each month.

TABLE III

COMPARISON OF PHENOL DEGRADATION BY AEROBIC AND ANAEROBIC CULTURES

	Phenol, ppm			
	Day 0	Day 8	Day 8*	Day 16
Anaerobic	72	68	56	48
Aerobic	72	0.6	18	0.1

*Immediately after sampling on the eighth day, half of the culture volume was discarded, the volume again made up with new wastewater, and another set of phenol analysis performed.

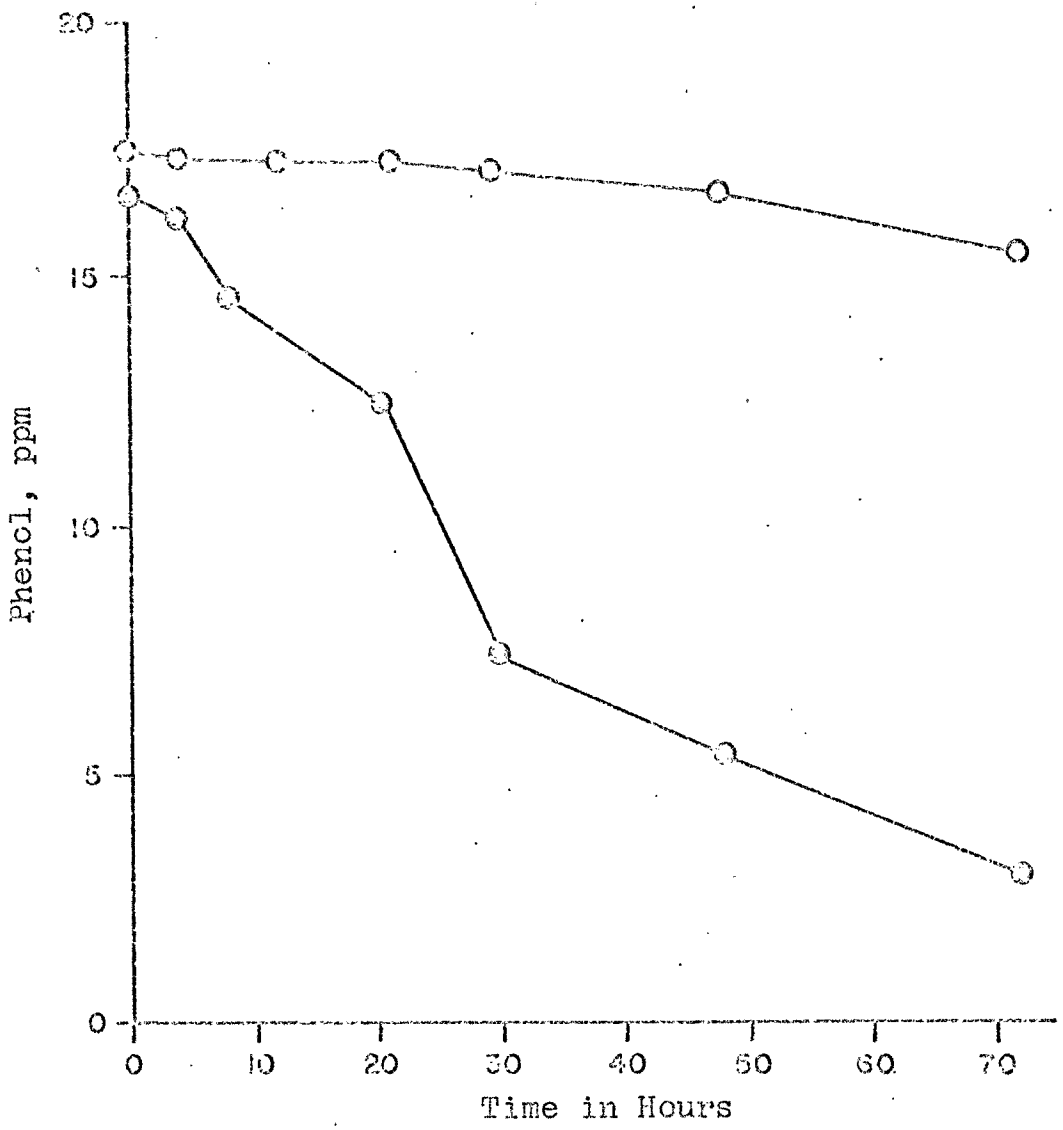

Fig. 5--The effect of a bactericidal agent on the phenol reduction rate in the plant effluent.

- O Phenol reduction rate following the addition of $HgSo_4$
- ◎ Phenol reduction rate of plant effluent without $HgSo_4$

addition of a bactericidal material, ($HgSo_4$) to the effluent, a sharp decline in the phenol reduction rate was observed, thus establishing the biological nature of phenol removal in the aerated effluent. This result was expected in that the data given in Table I and Table III and Figure 1, when considered together, indicate an equilibrium between the concentration of phenol in the water and the size of bacterial populations. Another consideration which affirms this view is that chemical removal of phenol generally requires stronger oxidative agents than air. The presence of phenol at all times in the effluent probably results from the lack of suitable growth conditions for the support of large numbers of phenol-utilizing bacteria.

A series of experiments were devised to investigate the phenomenon of aerobic metabolism of phenols in plant wastewater. The first series of experiments was designed to test the capability of the bacteria isolated from wastewater to degrade phenol, the second series to determine the necessity of adding nutrients to the plant wastewater in order to increase bacterial numbers and phenol removal rates, and the third series to investigate the effect of high concentrations of phenols on the efficiency of the system.

Tryptic Soy Eroth had been added in many of the previous experiments and acted as an added nutrient source for the bacteria. Effluent void of this nutrient source exhibited a slowed phenol removal rate which could be stimulated at any

time by the addition of Tryptic Soy Broth, as indicated in Figure 6. This same experiment implied that the phenol removal capabilities of the system were not enhanced by inoculation with the bacterial isolates. This is not surprising when one considers that the isolates were obtained from soils exposed to creosote or phenol contamination and that these same bacteria would probably be found in the polluted wastewater.

It was confirmed in past experiments that an added nutrient source increased the phenol reduction rate of the effluent. It was suspected that an upper limit of added nutrients could be obtained beyond which further addition would give no increase in the phenol removal rate. As indicated in Figure 7, additions greater than 5.0 per cent gave maximum reduction rates. It is inferred from these data that the wastewater lacked proper nutrients to support the bacterial populations necessary to bring about rapid removal of phenol. It is also evident that the addition of air to the culture system enhances phenol degradation. At the same time, it was not known whether or not a culture system of this type could withstand exposure to high concentrations of phenol and still yield adequate phenol reduction rates. Figure 8 indicates that by increasing the phenol concentration, the lag period prior to rapid phenol removal was also increased. This indicates that a continuous addition of large amounts of phenol to the effluent would result in the accumulation of toxic quantities of phenol and

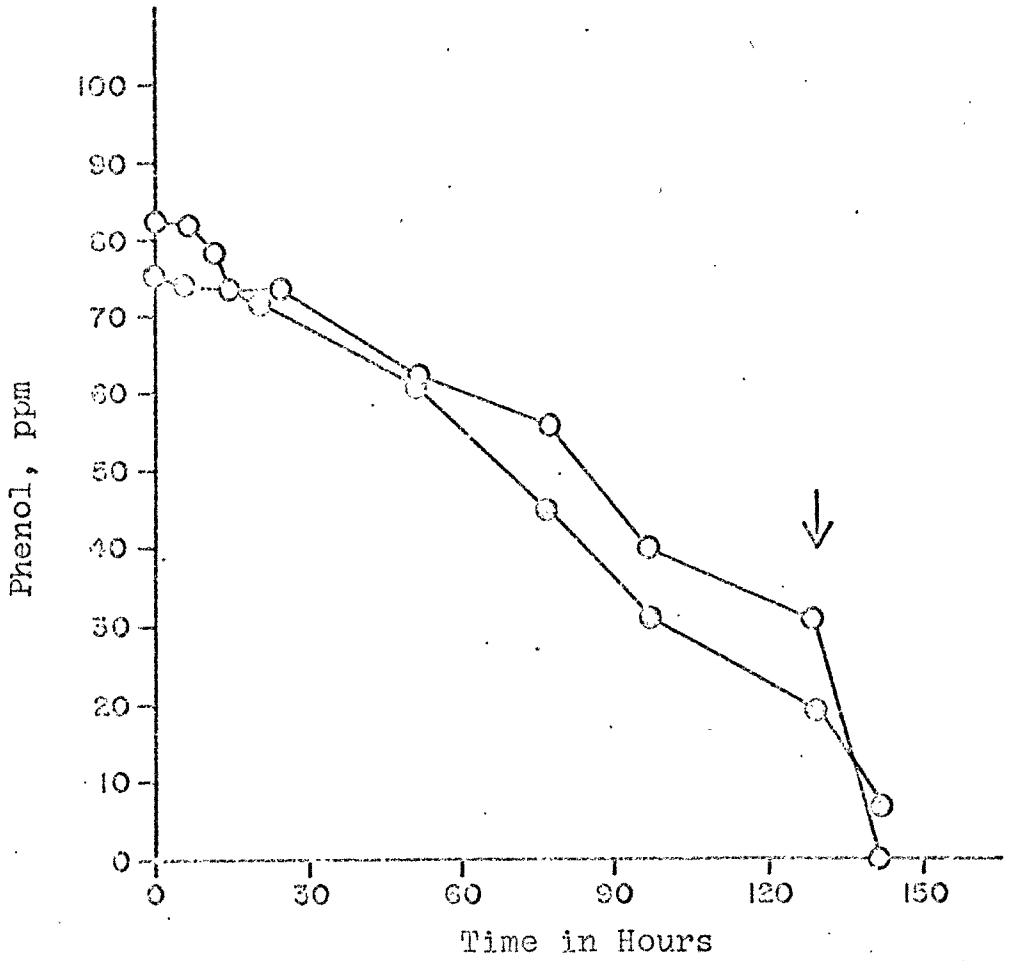

Fig. 6--The phenol degradation rate of inoculated effluent and the effect of Tryptic Soy Broth on the degradation rate.

Symbol: ◎ Sample uninoculated with mixed culture of isolates;
○ inoculated sample.
↓ Addition of Tryptic Soy Broth

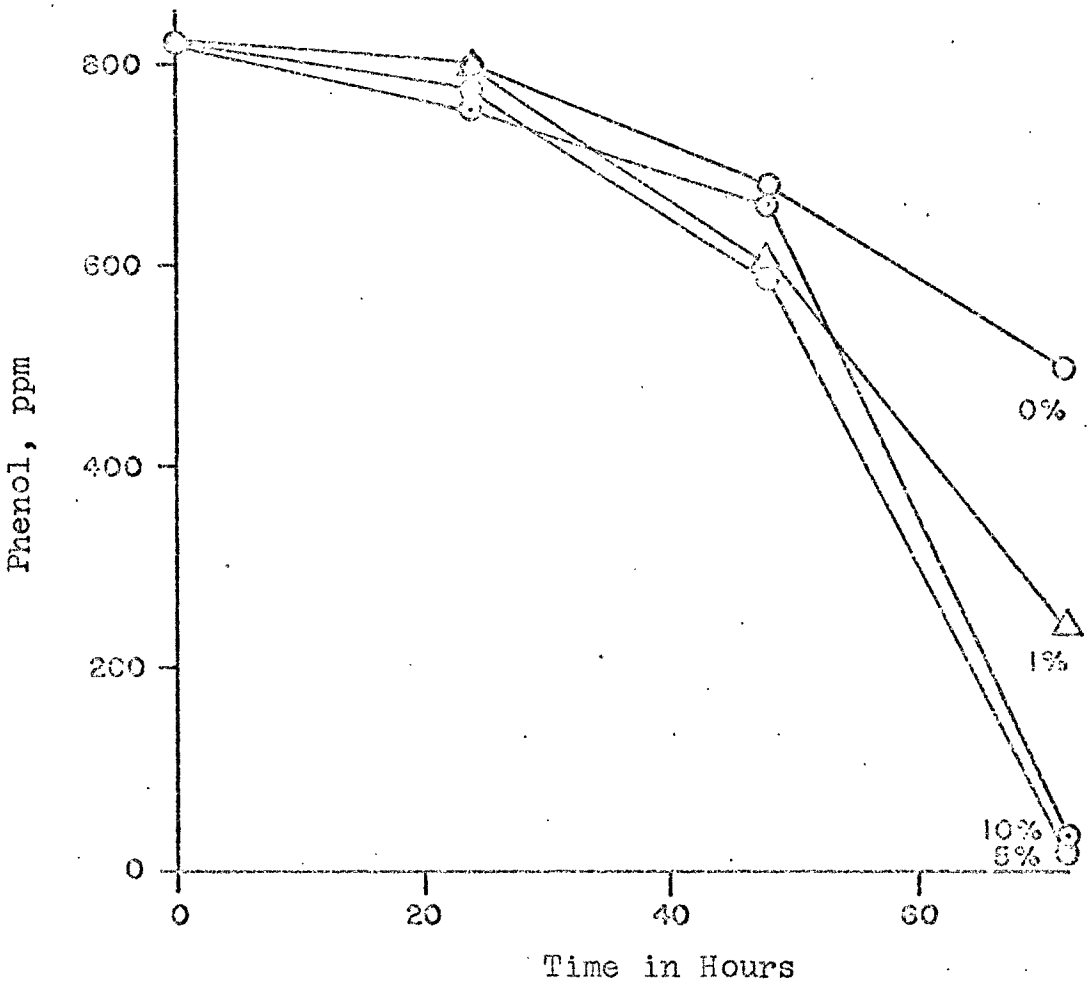

Fig. 7--The effect of variations in the Tryptic Soy Broth (% v/v) concentration on phenol reduction rate.

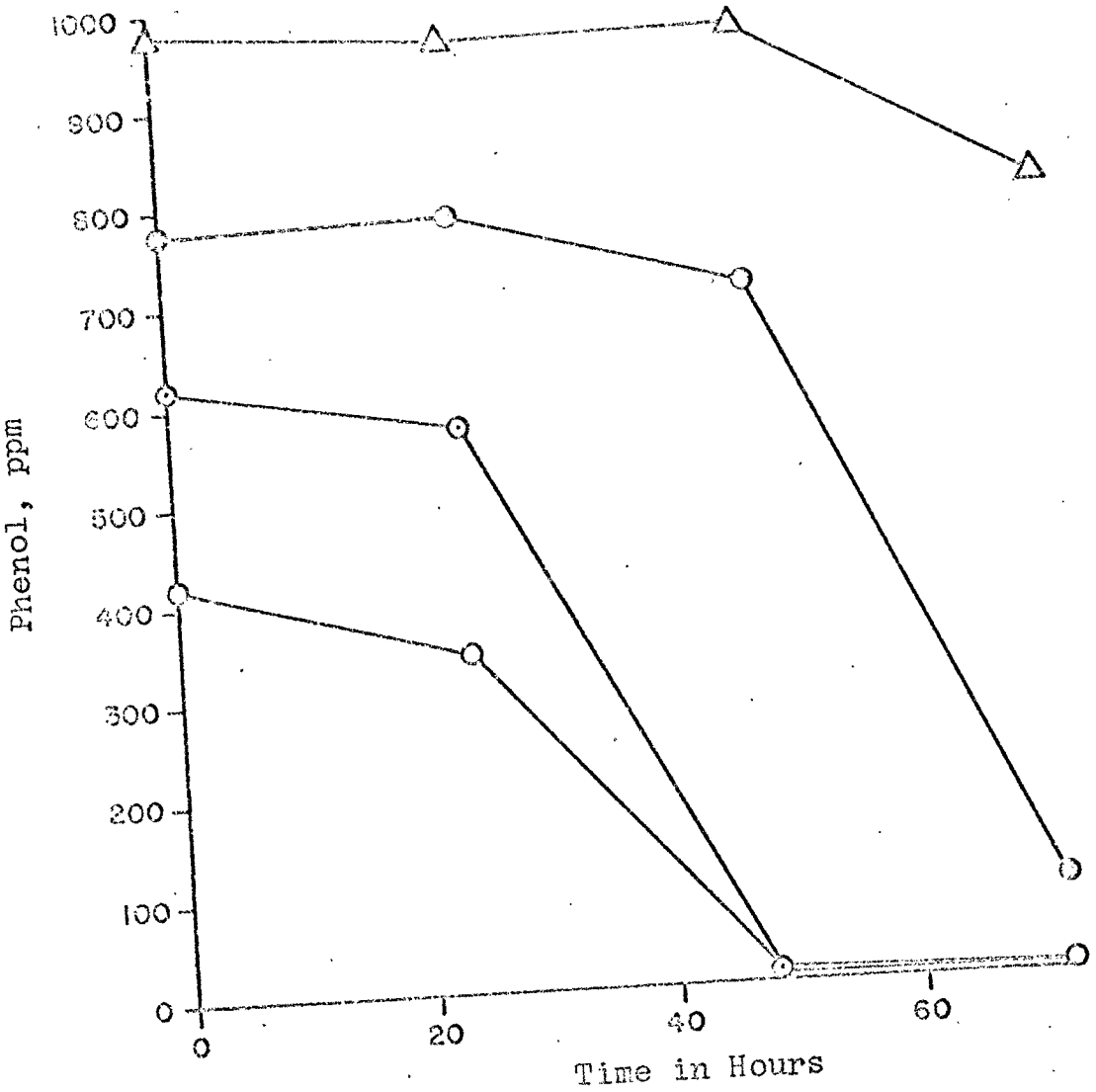

Fig. 8--Effect of high concentrations of phenol on the phenol reduction rate.

thus disrupt the entire system. This is not surprising when consideration is given to the fact that one of the best bactericidal substances is phenol dissolved in water to a concentration of 5 per cent (w/v).

The aforementioned experimental results were obtained utilizing Tryptic Soy Broth as a growth supplement, and therefore are useless in an industrial sense unless an economical supplement for Tryptic Soy Broth can be obtained. Ammonium phosphate was found to supply a suitable nitrogen and phosphorus source and, as indicated in Figure 9, compared favorably to Tryptic Soy Broth in its ability to enhance the phenol reduction rate of the culture. As indicated in the figure, 5 per cent ammonium phosphate (w/v) reduced the natural ability of the microbial flora of the wastewater to degrade phenol. A second experiment showed that 0.1 per cent yielded a more rapid phenol degradation rate than 1 per cent. This suggested that any amount of additive greater than that required for supplementing the culture medium of the bacteria acted detrimentally to the metabolism of phenol.

Therefore, it seems reasonable to state that the conditions for optimal phenol degradation can be established by adding a small amount (0.1 per cent w/v) of ammonium phosphate, and excess air.

The final experiment was concerned with resolution of the obvious effect of phenol concentration on metabolic activity

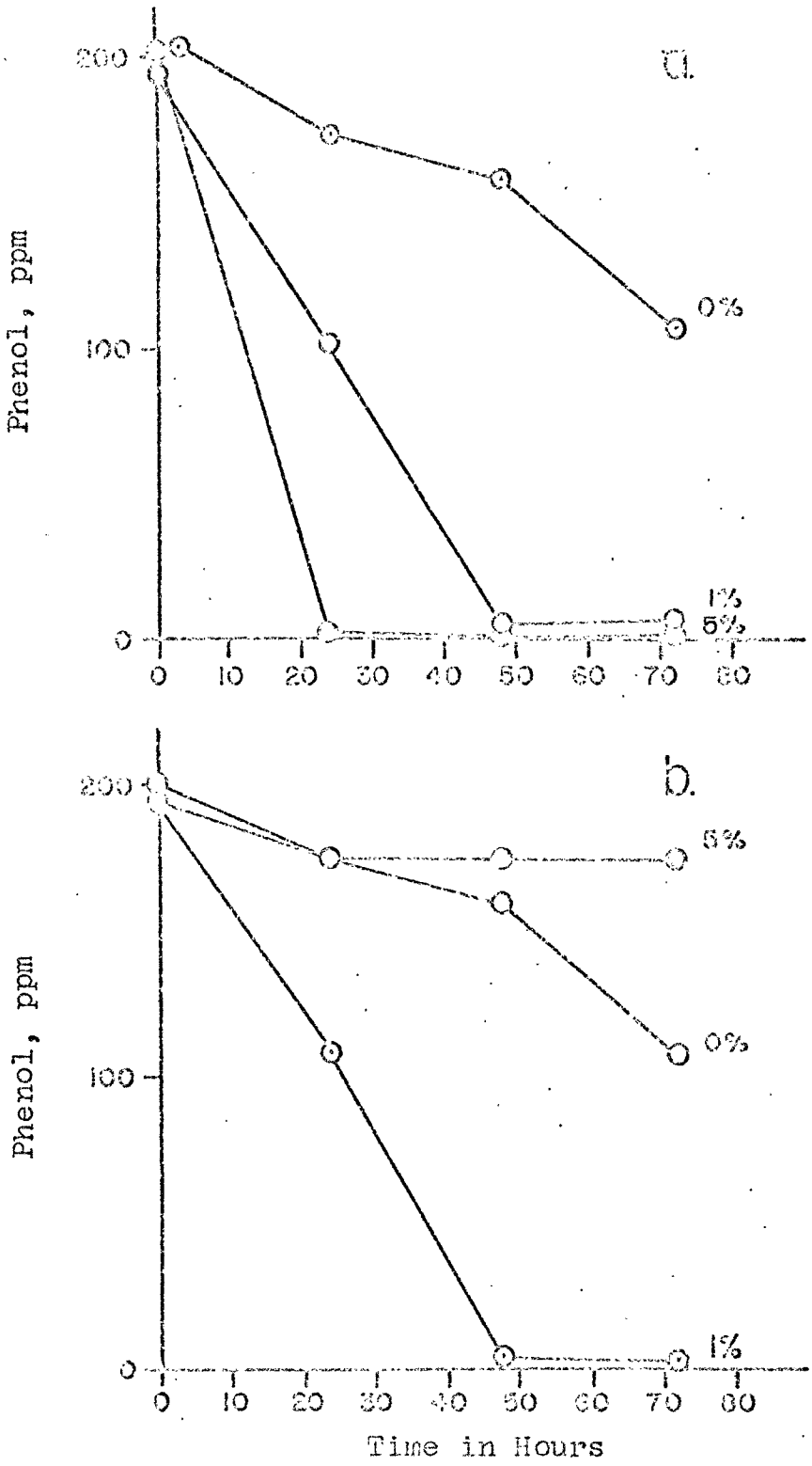

Fig. 9--Comparison of phenol degradation in identical cultures of wastewater to which different concentrations of Tryptic Soy Broth (a) and different concentrations of ammonium phosphate (b) have been added.

of the culture system. The data in Figure 8 show that if 1000 ppm phenol were added to the culture, a prolonged lag phase was created in comparison to cultures where only 400 ppm had been added. In further comparison, it is obvious that in the latter case, total degradation can be obtained in 40 hours under the conditions of the experiment, while in the former, only 20 ppm had been degraded. The significance of this phenomenon is that periodic addition of phenol to the culture is possible only when low concentrations are added and not so when high concentrations are added, unless the periodicity reflects increased log time for degradation. This phenomenon was investigated, using the concept of "adaptation" of mixed cultures. It can be easily seen in Figure 10 that two cultures have been adapted to rapid utilization of high concentrations of phenol following repeated additions of increasing amounts of phenol to the culture. These data also show that natural microflora of the wastewater is more than adequate to degrade high concentrations of phenol when placed under the proper conditions.

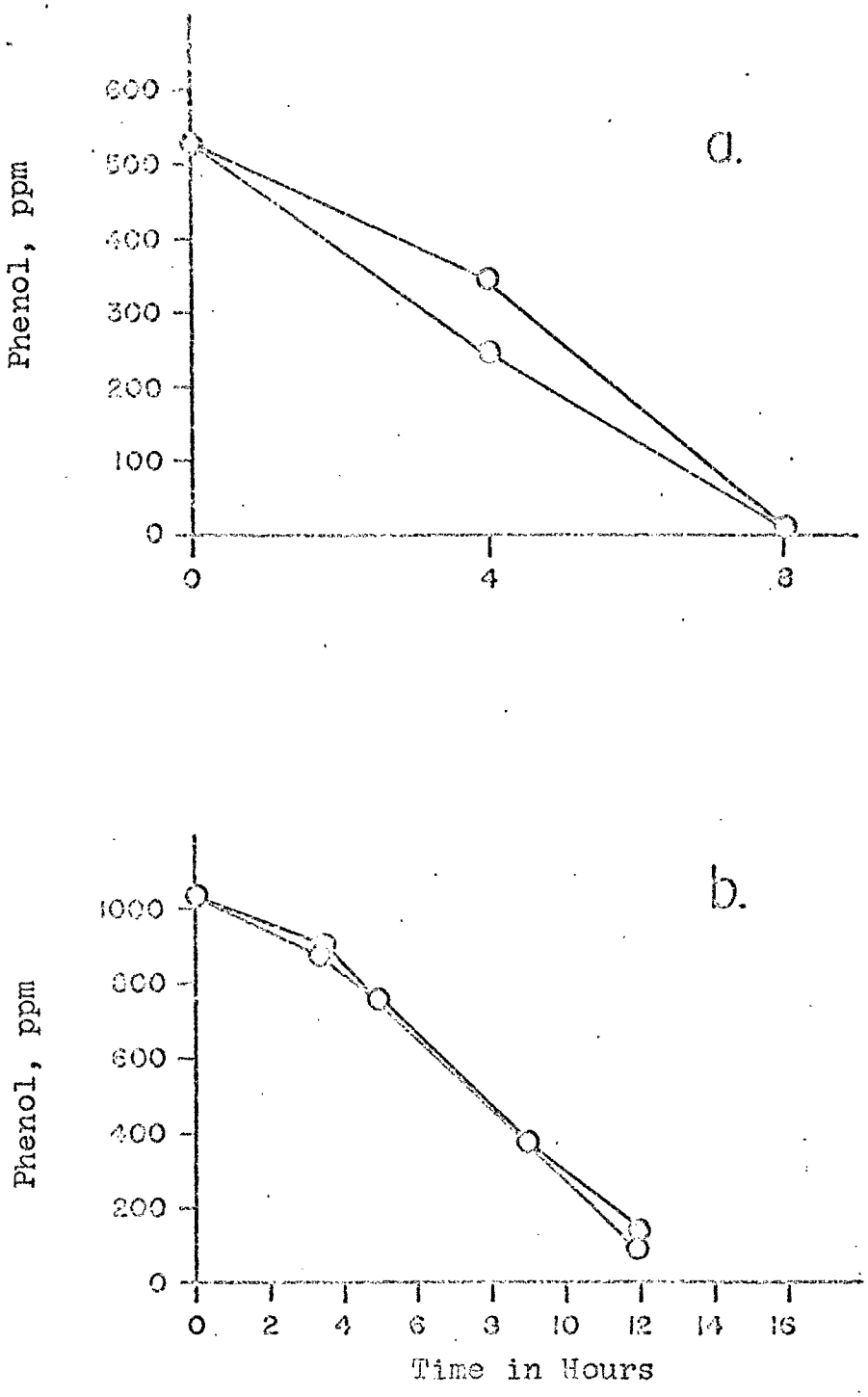

Fig. 10--Phenol removal rate of inoculated and uninoculated cultures following conditioning of the bacteria to high phenol concentrations: a, 500 ppm; b, 1000 ppm.
Symbol: ○ uninoculated effluent;
◍ inoculated effluent.

DISCUSSION

It was determined from the tests conducted on the plant effluent that pollution of the stream was heaviest during the colder months, possibly due to the lowering of biological activity during this period. The extent of pollution was assessed by the COD, BOD, and concentration of phenol.

One of the interesting, and somewhat surprising, results of this study was the very strong correlation between extent of pollution and water temperature. It is evident from this that the circumstances responsible for keeping high levels of COD, BOD, and phenol in the effluent water are affected drastically by water temperature. From this, it is reasonable to state that water purification is better accomplished at 30° C than, say, at 10° C.

From the experiments conducted, it was determined that an aerobic system was necessary for rapid removal of phenols. The laboratory data show unequivocally that phenol degradation occurs much more rapidly under aerobic conditions than under anaerobic conditions. It is inferred from the data presented that any system for wastewater purification based on bacteriological degradation will have to be designed for maximal aeration rates. Other design parameters which could be based on the research presented here would be pH, bacterial populations, phenol concentrations, and mineral constituents of the wastewater.

This research was designed on the premise that the majority of contaminants found in the wastewater from a creosoting plant are of natural origin or at least closely related to natural products. On this basis it seemed reasonable to assume that bacteria and other microorganisms in the soil would utilize them as nutrients. It was also assumed that soils and waters exposed to the creosoting plant effluent would be rich in these organisms. Many of the experiments conducted in the laboratory were based on these assumptions. The remainder were designed simply to find the conditions under which water purification could best be obtained. Some of the latter were conducted primarily to understand the nature of the purification process and only secondarily to obtain data for design.

The data obtained and the correlations discovered are in no way surprising. It can be frankly stated that almost all the findings were predictable from the point of view of classical microbiology philosophy. On the other hand, the actual quantitative determinations are novel and to an appreciable extent unique. For example, it could be predicted from basic concepts that temperature would affect pollutant degradation, but the finding that the phenol content of the wastewater emerging from the plant would be at least twice as high in December, January, and February as it was in July, August, and September came as something of a surprise.

The extent of this difference could not have been predicted or even approximated.

Another surprising fact which emerged from this research is the fact that the wastewater stream contained the exact microbiological populations necessary to bring about phenol degradation. While the concept of "self purification in nature" is well understood, it is generally thought of as a slow and very inefficient process. It is well known that the microorganisms capable of degrading organic substances in natural aqueous systems are generally present in those systems, but they are there in very small numbers and their metabolic activities are limited. It is surprising, therefore, to find that phenol degradation by the natural microflora is just as efficient as that of the best cultured bacteria available. (Please refer to Fig. 6.)

Since it was found that the mixed cultures of selected bacteria could survive in relatively high concentrations of phenol (400 ppm) or creosote (3,000 ppm v/v), it was deemed important to find the limit of this resistance. Again, prediction from general concepts would have placed the upper limit at some relatively low figure. In actuality, the laboratory experiments showed that the mixed cultures of laboratory bacteria as well as the natural populations found in the wastewater could survive in high concentrations of phenol. Several experiments showed that these bacteria can survive in

wastewater containing 1000 ppm phenol. In fact, the most important finding of this research is that if the proper conditions are maintained, the bacteria can degrade this quantity of phenol in a period of 12 to 14 hours.

CONCLUSION

In a plant effluent heavily contamined with creosote and phenols, purification may occur through a system of physical and biological occurrences. Creosote and oil removal is dependent on physical separation due to their relative weights. Phenols may be removed biologically if the composition of the effluent can sustain the growth of phenol-utilizing microorganisms. If the organisms are present but incapable of removing the pollutant, it may be necessary to amend the system so that microbial growth is possible; and water purification is then likely to result. This was the case in the plant effluent studies. Even though phenol-degrading bacteria were present in the plant effluent, their metabolic activities were insignificant until air and several nutrients were added.

From this situation develops a pattern that is applicable in many other situations of water pollution. That pattern would be recognition of the problem, isolation of organisms capable of removing or utilizing the pollutant, supplementation of the effluent to support the growth of the isolates, and inoculation and monitoring to determine the effectiveness of the system. This approach could be utilized in solving many pollution problems, but each problem is unique and requires individual research in approaching a solution.

LITERATURE CITED

1. Bayly, R. C., S. Dagley and D. T. Gibson. 1966. The metabolism of creosols by species of Pseudomonas. Journal of Biochemistry. 101 (6): 293-301.

2. Biczysko, Jan, and Jan Suschka. 1966. Investigations on phenolic wastes treatment in oxidation ditch. Journal of Water Pollution Control Federation. 38 (3): 366.

3. Chambers, Cecil W., Henry H. Tabak and Paul W. Kablor. 1963. Degradation of aromatic compounds by phenol-adapted bacteria. Journal of Water Pollution Control Federation. 35: 1517-1528.

4. Christensen, C. M., F. H. Kanfert, H. Schmitz and J. L. Allison. 1942. Hormodendrum resinae (Lindau), an inhabitant of wood impregnated with creosote and coal tar. American Journal of Botany. 29 (7): 552-558.

5. Davey, B. B. and Myfanwy Turner. 1961. Some phenol decomposing strains of Pseudomonas. Journal of Applied Bacteriology. 24 (1): 78-82.

6. Dagley, S., and M. D. Patel. 1956. Oxidation of p-cresol and related compounds by a Pseudomonas. Biochemical Journal. 66 (9): 227-233.

7. Deiters, A. M. 1956. Reports of information and technical development committee. American Wood-Preservers' Association. 52: 187-194.

8. Duncan, Catherine G., and Flora J. Deverall. 1962. Degradation of wood preservatives by fungi. Applied Microbiology. 12 (9): 57-62.

9. Eaton, G. C. 1957. Reports of information and technical development committee. American Preservers. 53: 215-220.

10. Eisenhauer, Hugh R. 1964. Oxidation of phenolic wastes. Journal of Water Pollution Control Federation. 36 (9): 1116-1128.

11. Eisenhauer, Hugh R. 1968. The ozonization of phenolic wastes. Journal of Water Pollution Control Federation. 40 (11): 1187-1899.

13. Gaudy, Jr., A. F., R. Scudder, M. M. Neeley, J. J. Perot and L. E. Crane. 1968. Studies on the treatment of wood preserving wastes. American Wood-Preservers. 64: 196-200.

14. Halff, Albert H. 1959. Slow sand filtration of wood treating plant wastes. American Wood-Preservers' Association. 55: 184-187.

15. Hall, D. A., and G. R. Nellist. 1959. Treatment of phenolic effluents. Journal of Applied Chemistry. 9: 565.

16. Ingols, R. S., P. Stevenson. 1963. Biodegradation of the carbon chlorine bond. Research Engineer. Vol : 4-8.

17. Jayasankar, N. P., and J. V. Bhat. 1966. Mode of attack on phenol by a micrococcus sp. isolated from coir rets. Canadian Journal of Microbiology. 12 (1): 1031-1039.

18. Kemper, J. H., and others. 1958. Wastewater disposal committee report. American Wood-Preservers' Association. 54: 188-190.

19. Kemper, J. H. and staff. 1960. Wastewater committee report. American Wood-Preservers' Association. 58: 201-204.

20. Kinney, John E. 1960. Evaluating the taste and odor control problem. Journal of American Water Works Association. 3 (4): 505-519.

21. Leadbetter, E. R. and J. W. Foster. 1958. Studies on some methane-utilizing bacteria. Archiv für Mikrobiologie. 30: 91-118.

22. Nakashio, Makio. 1969. Phenolic wastes treatment by activated sludge process. Journal of Fermentation Technology. 47 (2): 389-393.

23. Putilina, N. T. 1959. Microbes used in industrial purification installations for removal of phenols from waste H_2O. Mikrobiologia. 28 (5): 703-707.

24. Rao, I. P. S. Prasad, P. Murahari Rao, D. Seethapathi Rao, and G. K. Seth. 1969. Pilot plant studies on treatment of phenolic wastes by high-rate deep trickling filter. Environmental Health Vol. 11: 366-377.

25. Ribbons, D. W. 1966. Metabolism of o-cresol by Pseudomonas aeruginesa. Journal of General Microbiology. 44: 221-231.

26. Roche, J. N., and Staff. 1970. Report of committee ITD-2, waste water disposal. American Wood-Preservers' Association. 66: 225-231.

27. Smith, R. M. 1963. Some systems for the biological oxidation of phenol-bearing waste waters. Biotechnology and Bioengineering. 5: 275-285.

28. Spicher, R. G., and R. F. Skrinde. 1965. Effects of potassium permanganate on pure organic compounds. Journal of American Water Works Association. 57 (4): 472-483.

29. Standard Methods for the Examination of Water and Wastewater. 1965. 12th ed. American Public Health Association, Inc. New York.

30. Steenson, F. L., and N. Walker. 1957. The pathway of breakdown of 2:4-Dichloro and 4-Chloro-2-Methylphenoxyacetic acid by bacteria. Journal of General Microbiology. 16: 146-155.

31. Walker, N. 1954. Preliminary observations on the decomposition of chlorophenols in soil. Plant and Soil. 2 (2): 194-204.

32. Welsh, Gene G., and Jerome F. Thomas. 1960. Significance of chemical limits in USPHS drinking water standards. American Water Works Association. 52 (3): 289-291.

33. von Frank, A. J. 1969. Water pollution control in the wood preserving industry. American Wood-Preservers' Association. 67: 157-161.