A STUDY OF THE DEGRADATION OF SOME AZO DISPERSE DYES IN WASTE DISPOSAL SYSTEMS

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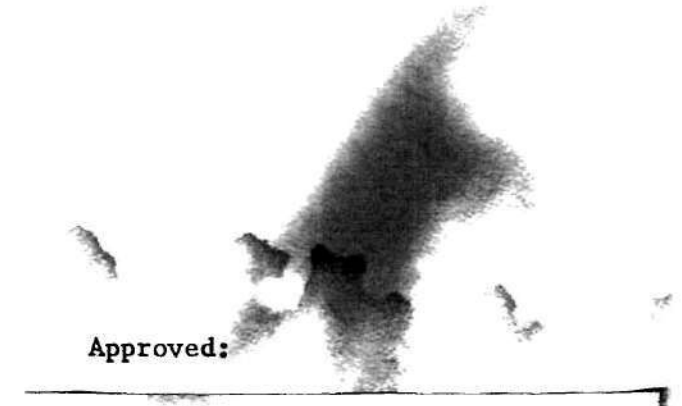

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SUMMARY

This study showed that two azo disperse dyes, Disperse Orange 5 (C.I. 11100) and Disperse Red 5 (C.I. 11215), were degraded by the bacteria in conventional waste treatment facilities into aromatic amines. Biological degradation produced identical metabolites as those formed by chemical reduction. Further modification of the amine corresponding to the p-phenylene diamine moiety was detected. It was found that dealkylation of the amine occurred resulting in p-phenylene diamine. The low oxygen uptake by the bacteria and apparent toxicity are discussed.

CHAPTER I

INTRODUCTION

This study was conducted to determine if certain disperse dyes used by the textile industry are toxic to the bacteria in waste treatment plants.

Disperse dyes are used to dye many of the synthetic fibers commonly used today. These fibers are hydrophobic and require dyes which are almost insoluble in water. However, these dyes have very high tinctorial value, and color the water, giving streams and rivers an unattractive appearance.

Many of the common commercial disperse dyes are of the azo type. Since the azo group does not occur in nature, it is possible that bacteria may have difficulty degrading this group. There is evidence that azo reduction occurs within the liver of higher animals producing amines and phenolic metabolites (30). Some bacteria contain the same enzyme system common to higher animals which is required to reduce the azo group. Therefore, this investigation was conducted to determine if the bacteria could degrade the dyes successfully.

The first studies on toxicity of azo compounds were reported in 1906 by Fischer (13) who caused a temporal hyperplasia of the epidermis by injecting scarlet red into the ear of a rabbit. Yoshida studied the chronic effect of a long term azo dye feeding and succeeded in producing the first hepatocarcinogenesis using orthoaminoazotoluene (41, 42).

Coal-tar carcinogenesis had been discovered some 17 years earlier by Yamagiwa and Ichikawa (40), but amino-azobenzene carcinogenesis is quite specific as to organs. Unlike coal-tar carcinogenesis, the azo dye induced only hepatomas regardless of the way it was administered, either orally, intraperitoneally, or subcutaneously (43, 28, 22).

Other substituted 4-amino-azobenzene dyes have also been found to produce hepatomas in laboratory animals. Among the more toxic dyes are 4'-fluoro-N,N-dimethyl-amino-azobenzene and 3'-methyl-N,N-dimethyl-4-amino-azobenzene (27).

The induction of hepatomas in laboratory animals by the administration of azo dyes has stimulated extensive studies for many years. These studies have included relationships between structure and carcinogenicity (18), metabolic fates of the dyes (31), and possible degradation mechanisms (26).

One study showed that carcinogenic activity was associated with dyes having critical density of electrons around the azo link using the Hammett substituent constant as a measure of the electron density (7). The dyes which showed carcinogenicity were also ones which had substituents in the 2' and 3' positions of 4-amino-azobenzene and these dyes all possessed the critical electron density around the azo link.

Many commercially available azo dyes have chloro substituents in both the 2' and 6' positions as well as having a 4'-nitro substitution. Studies by the Millers (27) showed that a nitro group in the 4' position did not cause hepatomas whereas 2' and 3' chlorine substitutions did show significant carcinogenicity.

The commercial dyes which have these or similar structures are disperse dyes used mainly to dye acetate and range in color from yellow to violet. Most often these azo dyes are various n-alkyl substituted 4-amino-azobenzene derivatives. The terminal hydroxyl of the alkyl group makes these dyes somewhat water soluble.

There have been few articles about the degradation of azo dyes by living organisms, yet there is much interest in the carcinogenicity of these dyes. One investigation about the degradation of azo dyes by microorganisms led to the discovery of sulfa drugs (10). Prontosil is enzymatically reduced to sulfanilic acid which inhibits the growth of microorganisms (35). Another investigation showed that this same reduction system can reduce other azo dyes and seemed to show no specificity as to structure (14).

Soil organisms showed the ability to degrade aromatic hydrocarbons such as phenol and its derivatives (34). This study showed that both chloro and nitro substitutions markedly increased the resistance to degradation. Both chloro and nitro substitutions are excellent chromophores and provide methods of intensifying and changing the color of a dye.

Soil organisms have successfully degraded the herbicide 3'4'-dichloropropionanilide to 3,4-dichloroaniline, which they condensed to form 3,4,3'4'-tetrachloroazobenzene (2).

Bray, et al., (4) showed that chlorinated nitrobenzenes formed chlorinated azoxy compounds through the chloroaniline intermediates.

This would indicate degradation might proceed by a similar mechanism since most reactions in living systems are reversible.

Stevenson, et al., showed that N,N-dimethyl-aminoazobenzene is metabolized into the following products: p-phenylene diamine, p-amino-phenol, and the N-acetyl derivatives of these bases (33).

The addition to certain enzyme systems of the diamine corresponding to one-half of the N,N-dimethyl-amino-azobenzene molecule, N,N-dimethyl-p-phenylene diamine, results in strong inhibition of enzyme activity (20,21).

Elson found that N,N-dimethyl-p-phenylene diamine and other aromatic diamines are potent thiol enzyme inhibitors (12). If this component were formed by the reduction of N,N-dimethyl-4-amino-azobenzene, it might well interrupt important energy-yielding processes and lead eventually to cancer formation. In fact, this is one of the popular theories to explain azo dye carcinogenesis.

McLean showed that the cleavage products of Butter Yellow strongly inhibit the urea forming enzymes in the liver (23). Fox found that some of the ring-substituted derivatives of azobenzene were effective seed protectants in the control of the fungus Pythium ultimum (15).

Sulfur containing azo dyes have caused similar hepatomas in mice to those found from the administration of Butter Yellow. Increases in hepatic activity were noted from the injection of the skin carcinogen 7,12-dimethyl-benz(α) anthracene (8).

N-hydroxylation metabolites have been found for the amino stilbene dyes and for the carcinogens, benzidine and 2-naphthylamine. It was found that the N-hydroxylation metabolites were as effective in causing disorders as the parent compounds (1, 3, 36).

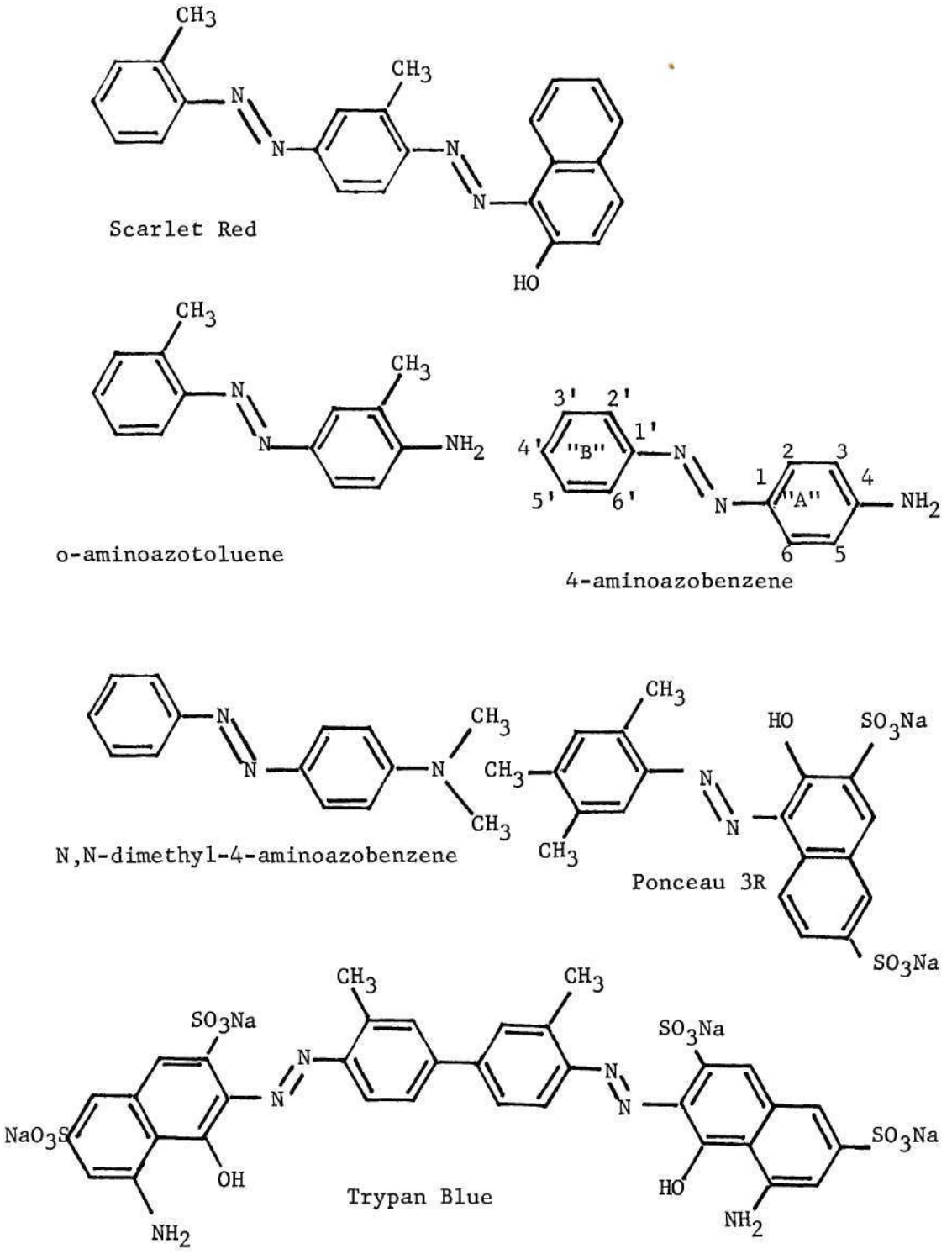


Figure 1. Structures of Some Carcinogenic Azo Compounds.

CHAPTER II

INSTRUMENTATION AND EQUIPMENT

Activated sludge was used as the method for degradation. This method of aerobic degradation is one of the most effective means of biological reduction of waste. This method uses a stream of air from the bottom of the tank to keep the sludge in continual motion. The model used for this study had the same design as the conventional aeration tanks used at waste treatment facilities (Figure 6).

Domestic sludge was collected from the aeration tanks at the Atlanta South River Treatment Plant. The sludge was fed from a 40 liter reservoir by gravity. The flow rate was one liter per hour and the capacity of the model was 13 liters. This provided for a 13 hour retention time before the effluent flowed over the weirs.

Samples were collected by attaching a rubber hose to the spout and allowing the effluent to collect in the sample vessel. This type of collection system facilitated the taking of a large sample. By using a 20 liter bottle to collect the effluent, the degradation of the dye could be studied over longer periods of time.

The infrared spectra of the metabolites and dyes was performed on a Beckman IR 10 recording spectrophotometer from both KBr pellets and chloroform.

The visible spectra of the effluent was monitored on Beckman DK-2A recording spectrophotometer using distilled water as a reference.

CHAPTER III

PROCEDURE

Preparation of Pure Dyes

Many articles have been written about the separation of commercial dyes using paper, thin layer, and column chromatography (5, 9, 11, 24, 32). The first two methods are mainly qualitative and only provide an indication of the purity. Column chromatography allows for collecting almost quantitative yields of pure dye in large quantities. The disadvantages of paper chromatography are the long period of time for development of the chromatogram, the small amount of sample which can be separated, and the destruction of the paper when harsh reagents are applied to identify invisible compounds.

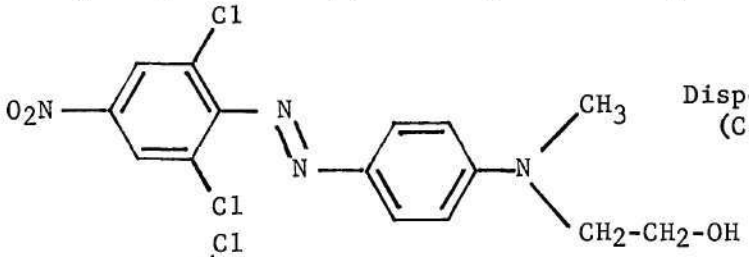
Thin layer chromatography was investigated as a method for determining the purity of the commercial dyes. This method was useful for selecting the manufacturer's dye which would produce the highest yield of pure dye.

> Individual trade products of various dye manufacturers are frequently identical, a fact easily shown by chromatography. The individual dyes, however, differ from each other in purity. The reaction mechanisms of dye synthesis is of a complicated and manifold nature. For this reason the impurities occurring in the dye very frequently consist of other dyes or intermediates formed during the course of the reaction. The nonuniformity of the dye mostly depends on the degree of purity of the intermediates, and on the tautomerism of the products likely to be isomers. Unreacted intermediates, isomers and other impurities must reportedly be the main cause of the nonuniformity of dyes.

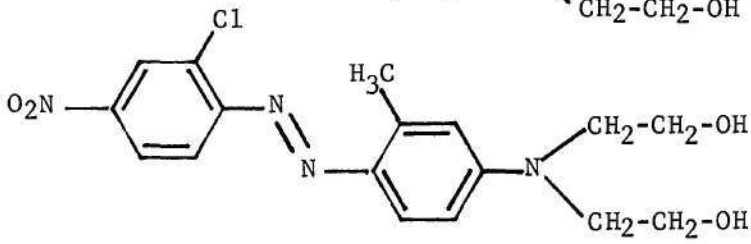
> Dyes are either pure homogeneous, i.e., they are free from any secondary dye impurities which might have been formed in the course of the dye synthesis itself, or shaded, i.e., they contain a small amount of another dye so that the desired shade is obtained, or lastly miscellaneous, these are formed by intentional mixing of several fundamental dyes... (32).

The dyes of commercial importance which contain the 4-amino-azobenzene precursor, which have known chemical structures, are mainly acetate dyes. Other hydrophobic fibers may be dyed with these dyes and they are suggested as the "work horse" colors for topping off shades.

Of this class the ones selected for this study were Disperse Orange 5 (C.I. 11100), and Disperse Red 5(C.I. 11215).



Disperse Orange 5
(C.I. 11100)



Disperse Red 5
(C.I. 11215)

Several methods for preparing alumina columns were investigated. The slurry method proved to be least satisfactory as the separations were not sharp and much tailing persisted. The method which proved to give sharp bands and good separation of the components was to add alumina slowly to the column filled with the eluting solvent and at the same time tapping the sides. This method provided a column of almost uniform consistency.

All the dyes chosen for this investigation were separated and purified from the commercial dyes in the same manner. Five grams (gm.) of the commercial dye were placed in a 1,000 milliliter (ml.) Erlenmeyer flask and boiled with 500 ml. of 95 percent ethyl alcohol. The liquor was filtered through a steam-jacketed Buchner funnel and evaporated on a Rotavapor vacuum distiller. The dye was redissolved in 750 ml. of methyl alcohol, transferred to a four liter Erlenmeyer flask, and boiled with two liters of distilled water. Distilled water was added dropwise to the boiling liquor until the solution clouded. The flask was wrapped with a towel, covered with aluminum foil and set aside to cool.

The cooled liquor was filtered through a Buchner funnel, and the crystals or plates were collected and stored in brown glass bottles.

By first dissolving the commercial dye in ethanol, the dye was separated from the dispersing agents and fillers. The crystallization from water and methanol removed any residual dispersing agent. This method also removed virtually all the starting intermediates.

One gram of the recrystallized dye was dissolved in the solvent used for elution of the alumina columns. The columns were one and one-half inches glass I.D. by 48 inches and packed with aluminum oxide (pH of slurry at 25° C., 7.8). The purified dye fraction was evaporated to dryness on the Rotavapor, the dye redissolved in methanol and recrystallized.

Acetone was the most effective solvent for separating Disperse Orange 5; whereas Disperse Red 5, being a diol, was strongly held to

the alumina and required a more polar solvent. A mixture of methanol and acetic acid (4:1) effectively eluted the dye from the column.

Both 5 x 20 cm. and 20 x 20 cm. thin layer plates were prepared with 30 gm. of E.R. Merck silica gel G and 60 ml. of distilled water. The slurry was vigorously swirled for one minute and applied with a Desaga spreader at a thickness of 250 microns. The plates were allowed to air dry at room temperature for 45 minutes and then activated at 120° C. for one hour. The plates were stored in a desiccator. Unless otherwise stated, the thin layer plates were spotted (20 mm.^{2}) 1.5 cm. from the bottom of the plate using the Desaga applicator with a micro-pipette. The chromatogram was developed 15 cm.

Isolation of Metabolites

All the metabolites were shown to have identical R_f values and identical infrared spectra with synthetic specimens when color was insufficient for confirmation. 2,6-dichloro-4-nitroaniline and 2-chloro-4-nitroaniline were obtained from Eastman Organic Chemicals. Each sample showed trace contamination by the other base. Purification of the bases was accomplished using an alumina column six inches by 1/2" I.D. and trichloroethylene was used to develop the column. This method separated the faster moving 2,6-dichloro-4-nitroaniline and the 2-chloro-4-nitroaniline was eluted with acetone. The bases were then recrystallized from a mixture of acetone and small amounts of petroleum ether.

N,N-bis-β-hydroxyethyl-3-methyl-4-aminoaniline and N-methyl, N-β-hydroxyethyl-4-aminoaniline were prepared from the reduction of the

corresponding dye using neutral sodium dithionite and stannous chloride in an acetone and water solvent. The reduction mixture was extracted with a mixture of chloroform and diethyl ether (1:1). The solvent was dried with anhydrous sodium sulfate and evaporated to dryness on a water bath. The residue was redissolved in 100 microliters of acetone and spotted on a 20 x 20 cm. thin layer plate. The plate was developed with a mixture of benzene and benzyl alcohol (3:1) and the fluorescent band corresponding to the p-phenylene diamine moiety was scraped off the plates, extracted with acetone, and the solvent evaporated to dryness.

An alternate method was developed for isolation of larger amounts of the substituted p-phenylene diamine. The corresponding dye was reduced, extracted with solvent, and the solvent evaporated to approximately one ml. This was applied at the top of the small alumina column. After the chlorinated bases were eluted with acetone, the substituted p-phenylene diamine was eluted with methanol.

Toluene-2,5-diamine and p-phenylene diamine were obtained from Eastman Organic Chemicals as the hydrochloride. As these chemicals are very reactive, they were prepared each day.

N-acetyl derivatives were prepared using acetic anhydride. Twenty mg. of the bases were acylated with three ml. of acetic anhydride and two drops of concentrated sulfuric acid. After 15 minutes, water was added dropwise until the mixture clouded and then placed on a water bath until the mixture became clear. The N-acetyl derivatives formed white needles from boiling water.

The hydrazobenzene derivatives were prepared by adding sodium dithionite in acetone and water.

Biodegradation Studies

The sludge, collected at the Atlanta South River Treatment Plant, was acclimated to the laboratory conditions (20° C.) using 50 liters of a 1:1 mixture of glucose and peptone at a concentration of 300 parts per million (ppm.).

Daily records were kept on the dissolved oxygen (D.O.) and temperature using a Union Carbide Dissolved Oxygen Analyzer. The visible spectra was recorded from 360-900 millimicrons. To determine whether toxicity or a biostatic effect had developed in the effluent, a count of the viable bacteria was made on nutrient agar in Petri dishes at 10^4, 10^6 and 10^8 dilution of daily samples of the effluent from the aeration tank. The samples were taken over a five day period and the colonies counted after 48 hours incubation time at 37° C.

The dyes were submitted to degradation from a dispersion made by pouring acetone and a solution of the dye into distilled water. The initial concentration of acetone was equal to or less than 300 ppm. which served both as a source of food and as a means of increasing the solubility of the dye. This method of feeding approximated natural conditions although in actuality the dyes are kept in dispersion by a surfactant.

One liter of effluent was extracted with 100 ml. of a mixture of ether and chloroform (1:1). The extract was dried with anhydrous sodium sulfate and evaporated to dryness on a steam bath. The residue was redissolved in two to five ml. of acetone and stored in 85 x 15 mm. test tubes in a dark place.

When more metabolite was required for analysis, five liters of effluent was extracted, and the combined extracts concentrated as described. This sample was chromatographed on the small column or on a micro column 15 x 4 mm. I.D. using acetone as the initial solvent. When stronger solvent was required, the eluting solvent was changed after the last band had eluted. The solvents were changed to isopropyl alcohol, to methanol, and, if any bands persisted, to a 50 percent mixture of acetic acid and methanol.

Each band was collected in an 85 x 15 mm. test tube. Unless acetic acid was used as the elution solvent, 25 mg. of KBr was added and the solvent evaporated to dryness on a steam bath. The contents were dried in a vacuum oven at 10 mm. pressure and 60° C.

The infrared spectra of the metabolites were made from KBr compressed into circular pellets on a hydraulic press. If particular bands were missing or nondistinct, the sample was dissolved in anhydrous chloroform. This method provided a means to determine whether complexing of the functional groups occurred.

CHAPTER IV

DISCUSSION OF RESULTS

Metabolism of Disperse Orange 5

The R_f value is defined as the ratio of the distance traveled by the compound to the distance traveled by the solvent. It is determined by measuring from the center of the spot to the origin. The solvent ascends the thin layer plate by capillary attraction until it reaches the finish line at which time the plate is removed from the vapor chamber and the solvent allowed to dry.

The R_f values for the extract of Orange 5 degradation, taken from one liter of effluent on consecutive days for one week, showed that the bacteria had not degraded the dye. The only spot detected was the parent dye. Therefore,a five gallon sample of the effluent was taken and allowed to stand to determine whether the bacteria, given a longer period of time, could degrade the dye under anaerobic conditions.

After seven days a spot of light yellow-orange metabolite and another of bright yellow metabolite appeared on the thin layer plates. The first spot co-chromatographed with one of the chemical reduction products, which later was shown to be the hydrazobenzene derivative. The chemical constitution of the second spot is unknown. The solvent that produced the best separation of the parent dye from these metabolites was a mixture of benzene and benzyl alcohol (3:1) (Table 1.).

At the end of three weeks a fluorescent spot appeared (Figure 2), indicating that the bacteria had reduced the azo bond into sizable amounts of N-β-hydroxyethyl,N-methyl-4-aminoaniline and a smaller amount of 2-6-dichloro-4-nitroaniline.

Stevenson et al. (33), found p-hydroxyaniline in the urine of rats injected with large doses of Butter Yellow. Butter Yellow (N,N-dimethyl-4-aminoazobenzene) is enzymatically reduced into p-hydroxyaniline and p-phenylene diamine. Both of these bases were found modified as acetanilides.

Since the 4' position on both of these dyes has a nitro substitution, no para hydroxylation could occur without replacement as does Butter Yellow. Para hydroxylation is considered to be a detoxifying mechanism of the liver and appears to occur even when fluorine is substituted in the 4' position of Butter Yellow (39).

It was found that the fluorescent metabolite N-β-hydroxyethyl, N-methyl-4-aminoaniline appeared to have the same intensity after seven weeks' exposure to the bacteria as that for three weeks' time. This unusual fact indicated that this type N,N-dialkyl substitution must be complexed to the enzyme surface or resist degradation by some other means. It is known that N,N-dimethyl-4-aminoaniline inhibits carboxylase activity in yeast (21), and sulfhydryl containing enzymes (6). The inhibition of urease (23) is not as significant since in lower forms of life ammonia is excreted directly and not through the urea cycle.

A fluorescent metabolite that remained at the starting point was identified as aromatic amine. Upon diazotization, it appeared as a red color when coupled into 1-naphthylamine. The metabolite had the same R_f value as p-phenylene diamine. This fact indicated that dealkylation of the amine had occurred. N-β-hydroxyethyl-4-amino-aniline was synthesized to determine whether N-demethylation had occurred as the Millers reported for Butter Yellow (25). This base was not the metabolite as it had a much higher R_f value (Table 1).

The small amount of 2,6-dichloro-4-nitroaniline found in the effluent indicated that further modification of the base had occurred. Since acylation of the base removed the yellow color by the withdrawing effect of the acetyl group, the plates were sprayed with concentrated sulfuric acid and baked at 110° C. until the products appeared as charred spots. No metabolite was detected that had the same R_f value as N-acetyl-2,6-dichloro-4-nitroaniline.

Metabolism of Disperse Red 5

Disperse Red 5 was found to have a solubility in water at 25° C. of 5 mg. per liter. This is 5 ppm., or about 20 times more soluble than Disperse Orange 5.

The color of a saturated solution of Disperse Red 5 at 25° C. is deep magenta. However, the Disperse Red 5 effluent from the aeration tank had a pale golden color. This rapid reduction in the color occurred within 24 hours and was significant since only a slight change occurred in effluent with Disperse Orange 5, even after two months.

The extract from four liters of effluent which had been exposed for 24 hours showed that the bacteria had reduced the azo bond into the hydrazobenzene derivative and cleaved the azo bond into aromatic amines.

Disperse Red 5 is reduced through the hydrazobenzene intermediate which is easily distinguished from the parent dye by its orange-red color (Table 2). The golden color of the hydrazobenzene disappeared on standing for 24 hours after the effluent came from the model. The resulting pale yellow color and slight fluorescence of the effluent under ultraviolet light showed that the complete reduction of the dye had occurred.

2-chloro-4-nitroaniline was found, but no acetanilide of the base could be detected (Table 2). It is apparent that the reactivity of the ring must be the determining factor for acylation of the amine. Both 2,6-dichloro-4-nitroaniline and 2-chloro-4-nitroaniline are very unreactive amines and require stronger conditions for acylation than p-hydroxyaniline found in Butter Yellow. It is this fact that could explain the absence of acetanilides in the effluent.

The significant difference in Disperse Red 5 was the absence of N,N-bis-β-hydroxyethyl-3-methyl-4-aminoaniline. Two fluorescent metabolites appeared near the starting point, and both formed a red color when coupled into 1-naphthylamine. This fact showed that they were aromatic amines. The method for detection was described by Gillo-Tos (17).

The similarity of the R_f values of both the fluorescent metabolite to that of toluene-2,5-diamine in solvent system S_1 (Table 2)

indicated that the bacteria had dealkylated the parent amine N,N-bis-β-hydroxyethyl-3-methyl-4-aminoaniline as was found for Butter Yellow(25). The rapid rate of dealkylation of this amine was such that it was not detected in the effluent; whereas, the similar amine N-β-hydroxyethyl, N-methyl-4-aminoaniline, which is a metabolite of Disperse Orange 5 (Table 1), was detected. Further dealkylation of the latter amine was accomplished in a much longer period of time. The metabolite with the R_f value of 0.00 co-chromatographed with Toluene-2,5-diamine and also appeared as a red spot using concentrated sulfuric acid spray (Table 2). The other fluorescent metabolite (R_f 0.06) was not identified.

Toxicity

To insure that no further degradation of the dye occurred, the samples of effluent with Disperse Orange 5 were filtered through a micropore filter which removed all the bacteria. The filtered samples were stored in a dark place and analyzed at one time to determine what changes had occurred. There was a shift in the maximum absorption region from 425-450 millimicrons to 390-420 millimicrons. This transition indicated that the color of the effluent was changing from orange to yellow, a fact only slightly detected by the naked eye. The spectra also showed that the bacteria were initially able to degrade the azo bond by the appearance of the yellow color, but after several days the orange color started to reappear and the sludge floc disappeared. The sludge floc reappeared within two to three days after the feeding of Disperse Orange 5 was discontinued.

The rapid rate of reduction of Disperse Red 5 in the aeration tank corresponded to the initial fall in the dissolved oxygen level and the

subsequent rise in D.O. correlated with the appearance of the aromatic amine metabolites (Table 3). The rate of increase in D.O. indicated that an inhibition might have occurred, since para substituted amines have caused the inhibition of urease (12). Toluene-2,5-diamine, which was identified as a metabolite, is similar to p-phenylene diamine which has been shown to inhibit succinoxidase (12). It is possible that such an inhibition occurred; thereby causing the low level of oxygen uptake.

The slow rise in the D.O., after the initial decrease in dissolved oxygen, (when Disperse Orange 5 was fed) was probably not an indication of toxicity as much as an indication of starvation due to the low amount of food added on a daily basis.

The number of colonies on the Petri dishes for all dilutions of Disperse Orange 5 effluent was almost the same. This unusual fact can be explained in two ways. It might have resulted from the breakdown of very small bacteria colonies during the mixing of the dilution water; or it was a sign that a biostatic effect was present and effective in the higher concentrations of the sample. If biostasis occurred, it would mean that the dilution of the toxic substances had allowed the number of bacteria in the samples of higher dilution to multiply at a level equal to that for the lesser dilutions. A similar type of inhibition or stasis occurs towards the antimetabolite p-amino-sulfonic acid.

The evaluation of toxicity was inconclusive. The low level of oxygen uptake indicated that the dye metabolites may have been toxic. A similar inhibition was found for similar degradation products of Butter Yellow (12).

CHAPTER V

CONCLUSIONS

Bacteria in conventional waste treatment facilities were able to reduce the azo bond in disperse azo dyes. There was a direct correlation between the number of water-solubilizing groups and the rate of biological degradation. An anaerobic condition was required for the reduction of the more hydrophobic dye. When the resulting aromatic amine had a nitro and/or chloro substitution, a residual yellow color persisted.

Color from one of the two pure dyes currently used in the textile industry was partially removed under conditions of test using activated sludge method of treatment. Partial biological degradation of the dye resulted in appearance of colorless toxic aromatic amine metabolies with degradation of the sludge. This fact indicated that the presence or absence of color is not an adequate criterium for determining the seriousness of pollution from textile dyeing operations aside from the esthetic aspect of color.

CHAPTER VI

RECOMMENDATIONS

This study showed that specific azo dyes used by the textile industry are biodegraded into aromatic amines. It is suggested that other azo dyes be investigated to determine whether they biodegrade into aromatic amines. Trypan Blue (C.I. Direct Blue 14) and other disazo dyes have been shown to cause cancer and other disorders (16, 29).

The solution to color in textile mill effluent is not to build larger and more sophisticated treatment facilities, but to investigate the use of nonaqueous dyeing systems. Current technology about nonaqueous solvent systems has developed to the extent that feasible methods of solvent reclamation by distillation are available. This method of dyeing would eliminate the dyeing process in the textile industry as a source of pollution and significantly reduce the residual color left in water as the result of dye degradation.

APPENDIX

Table 1. R_f Values of Disperse Orange 5

and Its Possible Degradation Products

Compound	S_1	S_2	D_1	D_2	#
Disperse Orange 5	0.34	0.00	O		
N-β-hydroxyethyl,N-methyl-4-aminoaniline	0.37	0.00		+	+
2,6-dichloro-4-nitroaniline	0.95	0.49	Y		+
N-β-hydroxyethyl, N-methyl-2',6'-dichloro-4'-nitro-hydrazobenzene	0.28	0.00	Y-O		+
N-acetyl-2,6-dichloro-4-nitroaniline	0.85*				-
N-β-hydroxyethyl-4-aminoaniline	0.28			+	-
p-phenylenediamine	0.00			+	+

S_1 = 3:1 mixture (v/v) Benzene:Benzyl Alcohol

S_2 = Trichloroethylene

D_1 = visible

D_2 = fluorescence under ultraviolet light

* = charred spot with concentrated sulfuric acid

\# = compounds identified as metabolites

O = Orange

Y = Yellow

Table 2. The R_f Values for Disperse Red 5 and Some of Its Possible Degradation Products

Compound	S_1	S_2	D_1	D_2	#
Disperse Red 5	0.30	0.00	P		
N,N-bis-β- hydroxy-ethyl-4-amino-3-methyl-aniline	0.34	0.00		+	-
2-chloro-4-nitro-aniline	0.87	0.17	Y		+
2-methyl-N,N-bis-β-hydroxyethyl-2'-chloro-4' nitro-hydrazobenzene	0.31	0.00	R-O		+
N-acetyl-2-chloro-4-nitroaniline	0.75*				-
Toluene-2,5-diamine	0.00			+	+

S_1 = 3:1 mixture (v/v) benzene:benzyl alcohol

S_2 = Trichloroethylene

D_1 = visible

D_2 = fluorescence under ultraviolet light

* = charred spot with concentrated sulfuric acid

P = Purple

Y = Yellow

\# = compounds identified as metabolites

Table 3. The Dissolved Oxygen Level in the Aeration Tank

Disperse Orange 5*			
Day	Dissolved Oxygen**	Temperature °C.	Ratio to Saturation
1.	5.2	21.0	0.59
2.	5.0	21.5	0.57
3.	5.4	20.8	0.61
4.	5.8	20.4	0.66
5.	7.0	19.6	0.79
6.	7.7	19.8	0.86
7.	8.4	20.4	0.95
Disperse Red 5* **			
Day	Dissolved Oxygen	Temperature °C.	Ratio to Saturation
1.	6.2	19.6	0.68
2.	5.4	20.2	0.61
3.	7.7	20.0	0.87
4.	8.3	20.0	0.94
5.	8.4	20.2	0.95
6.	8.6	20.8	0.97
7.	8.7	20.6	0.98

* concentration 3 ppm.
** in parts per million
***concentration 3 ppm.

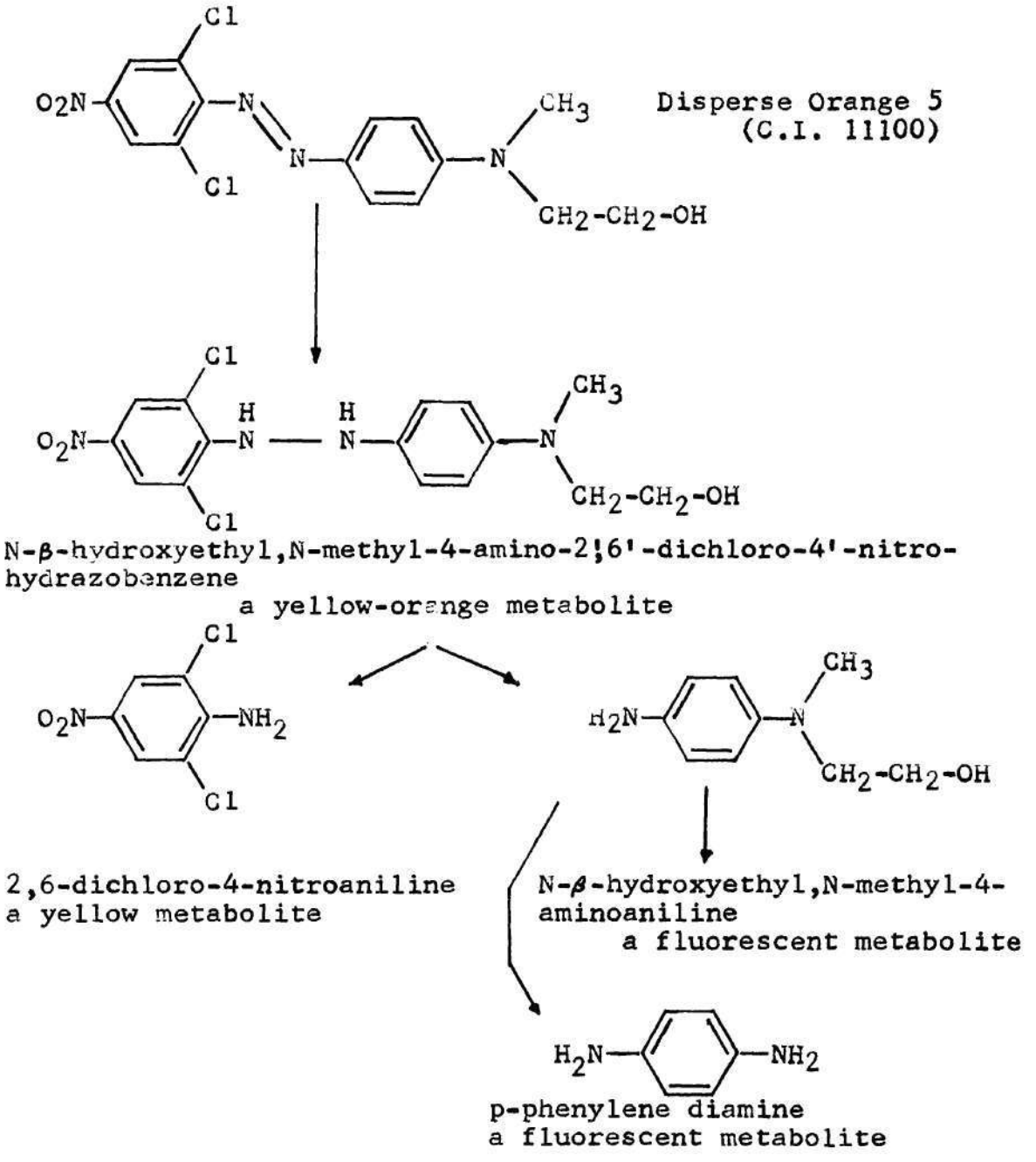
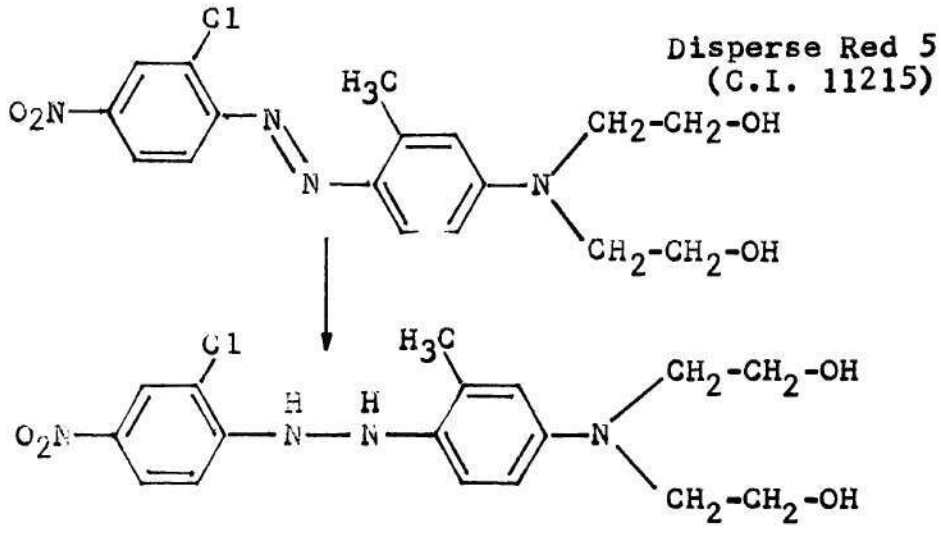
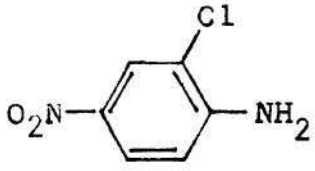


Figure 2. Degradation Pathway of Disperse Orange 5.



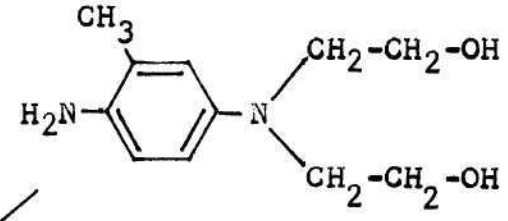
N,N-bis-β-hydroxyethyl-4-amino-2-methyl-2'-chloro-4'-nitro-hydrazobenzene

a red-orange metabolite



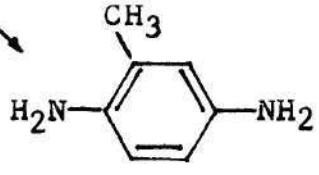
2-chloro-4-nitroaniline

a yellow metabolite



N,N-bis-β-hydroxethyl-3-methyl-4-aminoaniline

a fluorescent reduction product not found in effluent



Toluene-2,5-diamine

a fluorescent metabolite

Figure 3. Degradation Pathway of Disperse Red 5.

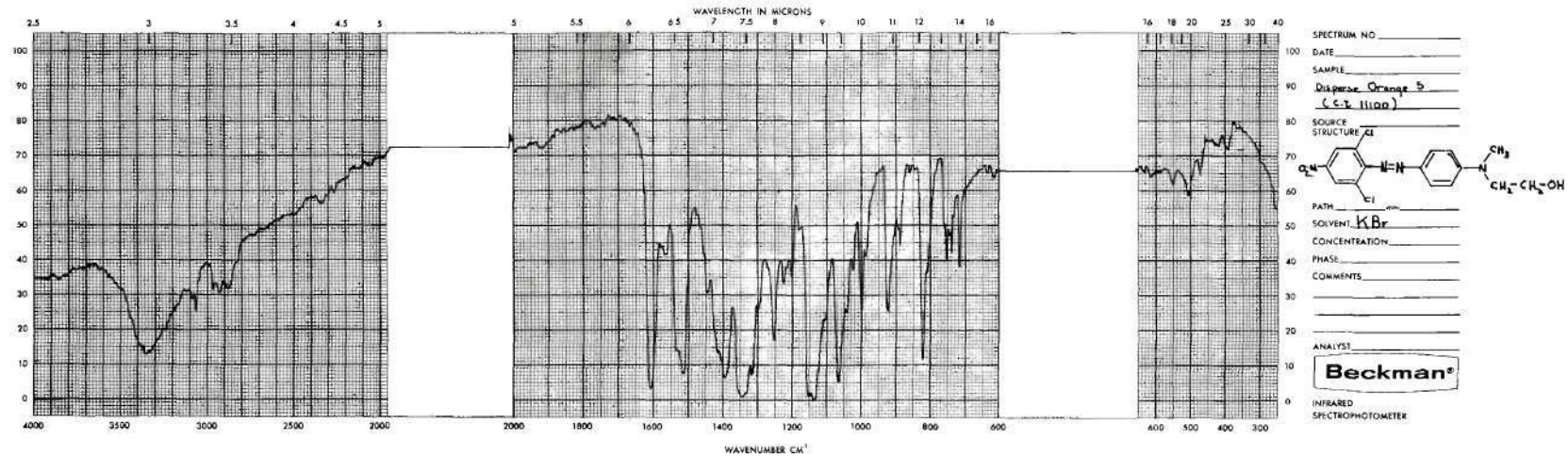


Figure 4. The Infrared Spectra of Disperse Orange 5

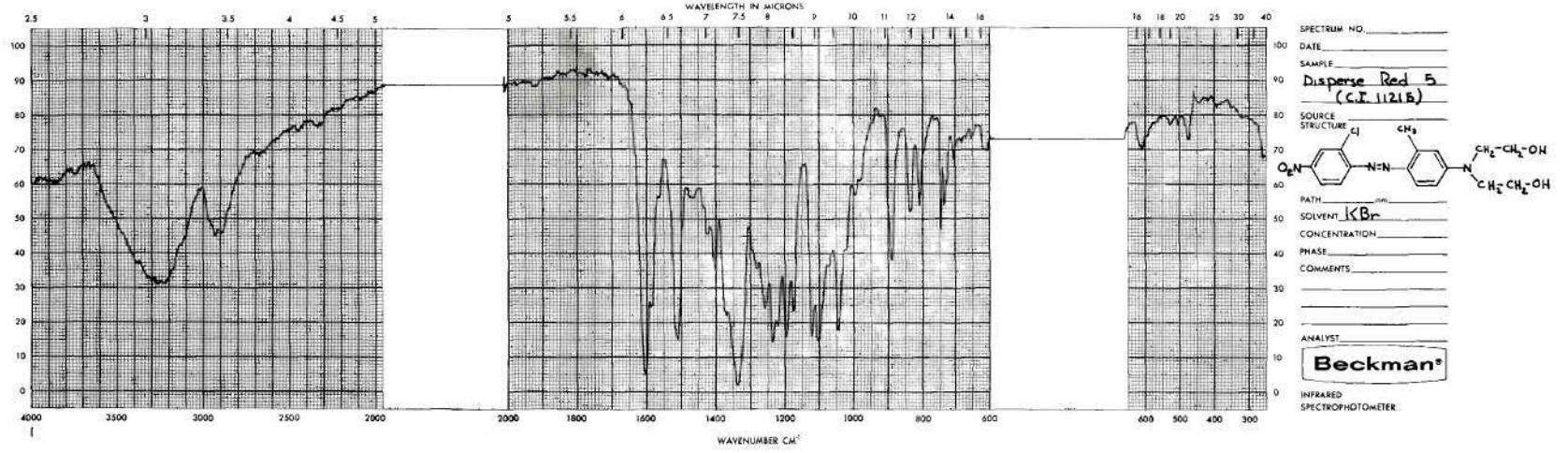


Figure 5. The Infrared Spectra of Disperse Red 5

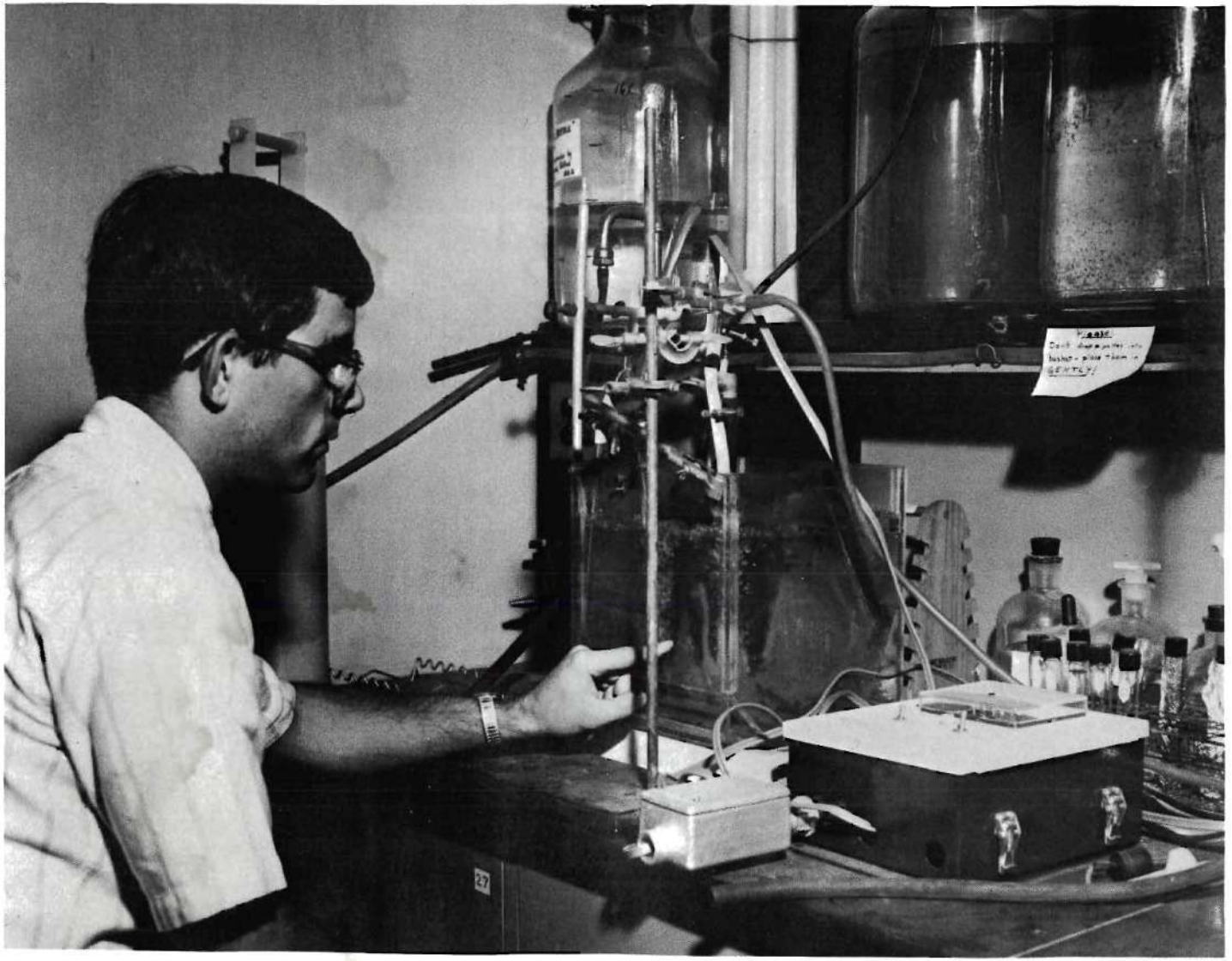


Figure 6. The Model Aeration Tank

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