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Project Summary

Fate of Water Soluble Azo Dyes in the Activated Sludge Process

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The objective of this study was to determine the partitioning of water soluble azo dyes in the activated sludge process (ASP). Azo dyes are of concern because some of the dyes, dye precurors, and/or their degradation products such as aromatic amines (which are also dye precurors) have been shown to be, or are suspected to be, carcinogenic. Specific azo dyes were spiked at 1 and 5 mg/L to pilot-scale treatment systems with both liquid and sludge samples collected. Samples were analyzed by high performance liquid chromatography (HPLC) with an ultraviolet-visible detector.

Mass balance calculations were made to determine the amount of the dye compound in the waste activated sludge (WAS) and in the activated sludge effluent (ASE). Of the 18 dyes studied, 11 compounds were found to pass through the ASP substantially untreated, 4 were significantly adsorbed onto the WAS, and 3 were apparently biodegraded.

This Project Summary was developed by EPA's Water Engineering Research Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The U.S. Environmental Protection Agency's (EPA) Office of Toxic Substances evaluates Premanufacture Notification (PMN) submissions under Section 5 of the Toxic Substances Control Act. Azo dyes constitute a significant portion of these submissions. Generally, azo dyes contain between one and three azo linkages (-N = N-), linking phenyl and naphthyl radicals that are usually substituted with some combination of functional groups including: amino ($-NH_2$); chloro (-Cl); hydroxyl (-OH); methyl ($-CH_3$); nitro ($-NO_2$); and sulfonic acid, sodium salt ($-SO_3Na$).

One aspect of the PMN review process is to estimate the release of a new chemical. The industrial manufacturing and processing of azo dyes will generate a wastewater contaminated with azo dyes, which is typically treated in a conventional wastewater treatment system. The effectiveness of this treatment must be known in order to estimate the release from this source. Therefore, EPA's Water Engineering Research Laboratory, Office of Research and Development undertook a study to determine the fate of specific water soluble azo dye compounds in the ASP.

The study was approached by dosing the feed to the pilot ASP systems with various water soluble azo dyes and by monitoring each dye compound through the system, analyzing both liquid and sludge samples. The fate of the parent dye compound was assessed via mass balance calculations. These data could determine if the compound was removed by adsorption, apparent biodegradation, or not removed at all. The report presents results for 18 dye compounds tested from June 1985 through August 1987. The study was

conducted at EPA's Test and Evaluation Facility in Cincinnati, OH.

Experimental Program

Screened raw wastewater from the Greater Cincinnati Mill Creek Sewage Treatment Plant was used as the influent (INF) to three pilot-scale activated sludge biological treatment systems (two experimental and one control) operated in parallel. Each system consisted of a primary clarifier (33 L), complete-mix aeration basin (200 L), and a secondary clarifier (32 L).

Each water soluble dye was dosed as commercial product to the screened raw wastewater for the two experimental systems operated in parallel at targeted active ingredient doses of 1 and 5 mg/L of influent flow (low and high spike systems, respectively). The principal focus of this work was on the ASP, and, as such, the primary sludge was not sampled. Table 1 presents a summary of the average operating conditions of the pilot-plant systems.

Before each data collection phase, dye analytical recovery studies were conducted using organic-free water, influent wastewater, and mixed liquor. These studies were run in duplicate and each recovery study was repeated at least once to ensure that the compound could be extracted from these samples. Purified dye standards were analytically prepared from the commercial dye product by repeated recrystallization.

The INF, primary effluent (PE), and ASE were filtered, and the filtrate was passed through a column packed with resin. The filter paper and resin were soaked in an ammonia-acetonitrile solution and then Soxhlet extracted with ammonia-acetonitrile. The extract was concentrated and brought up to 50 mL volume with a methanol/dimethyl-formamide solution. The mixed liquor (ML) samples were separated into two components, the filtrate or soluble (SOL) fraction and the residue (RES) fraction. The SOL fraction was processed similar to the INF, PE, and ASE samples. The RES fraction and the filter paper were processed similar to these samples but the resin adsorption step was omitted. All extracted samples were analyzed by HPLC with an ultraviolet-visible detector. Total suspended solids (TSS) analyses were also performed on the INF, PE, ML, and ASE samples.

All systems were operated for at least three times the solids retention time to ensure acclimation prior to initiation of data collection. All samples were 24 hr composites made up of 6 grab samples collected every 4 hr and stored at 4°C. The 18 water soluble, acid and direct azo dyes studied in pilot-scale ASP systems are listed below in Table 2 by Colour Index name and number. Figure 1 presents the chemical structure for each.

Results and Discussions

Before a compound was judged acceptable for spiking into the pilot-scale treatment systems, spike recovery studies were conducted for each dye. These tests were conducted using laboratory, organic-free water samples and several wastewater and sludge samples from the control ASP. All samples were spiked and held at 4°C for 24 hr before recovery was assessed. The possible removal mechanisms for a dye compound in the ASP system include adsorption, biodegradation, chemical transformation, photodegradation, and air stripping. Table 3 presents the results from these determinations. Recovery for most dyes was within the targeted range of 80% to 120%; thus, it appeared that little or no chemical transformation occurred for these dyes because of contact with the variable wastewater and/or sludge matrix under these conditions. Some recoveries from wastewater and/or sludge samples for four of the dyes were outside the targeted range, but these dyes were accepted for sampling because such recoveries were considered acceptable to the general project guidelines. As the recoveries for all 18 dyes were generally very good and with relatively low standard deviations, all values in Tables 3-5 are presented as measured and no correction made for recovery. In addition, no photodegradation of the dyes was found in laboratory studies. Moreover, the estimated Henry's law constant for each dye tested was less than 10^{-15} atm-m^3/mol, and, as such, air stripping was very unlikely. Therefore, adsorption and/or biodegradation appeared to be the only removal mechanisms.

Table 4 presents the mean concentrations for each of the dyes tested. Four dyes have two runs reported whereas all other dyes have just one. Additional runs were conducted for quality assurance/quality control reasons. From the results in Table 4 and TSS data, mass balance calculations can be made (see Table 5). If a compound in Table 5 was recovered near the targeted range of 80% to 120%, then it was assumed that this compound was not biodegraded since most of the compound was recovered. Conversely, if the recovery was less than 20% to 30% then it was assumed the compound wa apparently biodegraded. This assumptio was valid only because prelimina recoveries (Table 3) indicated little or n problems in recovering the compoun from the various sample matrices. Lastl if the compound was recovered near th targeted range of 80% to 120%, then or must investigate the percentag adsorbed data. If these data indicate less than 20% adsorbed, then it wa assumed that the compound wa substantially untreated by the AS However, if these data indicated that th amount adsorbed was greater than 30% then it was concluded that such compound was removed by appare adsorption.

Eleven of the 18 azo dyes studied Table 5 passed through the AS substantially untreated with the data fro the low and high spike systems excellent agreement for these dye These were:

- C.I. Acid Black 1
- C.I. Acid Orange 10
- C.I. Acid Red 1
- C.I. Acid Red 14
- C.I. Acid Red 18
- C.I. Acid Red 337
- C.I. Acid Yellow 17
- C.I. Acid Yellow 23
- C.I. Acid Yellow 49
- C I. Acid Yellow 151
- C.I. Direct Yellow 4

The relatively high sulfonic ac substitution of these dyes may expla why they were not removed. If the a dye has high sulfonic acid substitutio then little or no adsorption of the dye t the microbial cell or cell byproduc would occur, thus limiting the chance aerobic biodegradation. Ten of the 1 above dyes have at least two sulfon acid functional groups, C I. Acid Red 3 has one.

The positioning of the sulfonic ac functional group(s) and the molecul weight of the compound also appeared have an affect on how the compour partitions. Note in Table 5 that fo compounds were adsorbed onto th WAS and apparently not biodegrade These were:

- C I. Acid Blue 113
- C I Acid Red 151
- C.I. Direct Violet 9
- C I. Direct Yellow 28

C.I. Acid Blue 113, C I. Acid Re 151, and C I. Direct Violet 9 represe three of the four disazo (two azo bond structures Although these dyes a

Table 1. *Summary of Operating Conditions*

Parameter	Value
Influent flow rate, L/d	*720*
Primary sludge flow rate, L/d	*6*
Primary effluent flow rate, L/d	*714*
Mixed liquor wastage flow rate, L/d	*67*
Secondary effluent flow rate, L/d	*647*
Solids retention time, days	*2.7*
Hydraulic retention time, days	*0.28*
Dissolved oxygen, mg/L	*2.0-4.0*
Target influent spike dosages, mg/L	
Low	*1*
High	*5*
Influent pH, pH units	*7.0-8.0*
Aeration basin temperature, °C	*21-25*

Table 2. *Dye Compounds Spiked to the Activated Sludge Process*

Colour Index Name	Colour Index Number
C.I. Acid Black 1	*20470*
C.I. Acid Blue 113	*26360*
C.I. Acid Orange 7	*15510*
C.I. Acid Orange 8	*15575*
C.I. Acid Orange 10	*16230*
C.I. Acid Red 1	*18050*
C.I. Acid Red 14	*14720*
C.I. Acid Red 18	*16255*
C.I. Acid Red 88	*15620*
C.I. Acid Red 151	*26900*
C.I. Acid Red 337	*----**
C.I. Acid Yellow 17	*18965*
C.I. Acid Yellow 23	*19140*
C.I. Acid Yellow 49	*18640*
C.I. Acid Yellow 151	*13906*
C.I. Direct Violet 9	*27885*
C.I. Direct Yellow 4	*24890*
C.I. Direct Yellow 28	*19555*

**Not assigned as of 12/87. Chemical Abstracts Number 67786-14-5.*

sulfonated compounds with two of the three having two sulfonic acid functional groups, they also have a greater molecular weight than the other compounds. Further investigations into the affect of sulfonation (both in number of groups and position) versus molecular weight are necessary before a relationship, if any exists, could be developed.

Note also in Table 5 that three compounds appeared to be biodegraded. These were:

C.I. Acid Orange 7
C.I. Acid Orange 8
C.I. Acid Red 88

The conclusion that these compounds were apparently biodegraded comes from an inspection of the mass balance data; for each compound, very little of the dye was recovered during sampling. However, the preliminary recovery studies showed that the compound could be recovered without difficulty from wastewater and sludge matrices (see Table 3). Since the compounds were not found in the ASE or ML samples and chemical transformation appeared not to be occurring, then biodegradation would account for the loss of the parent compound.

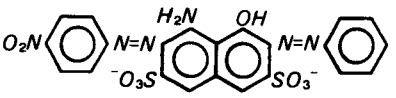
In addition to the 18 dyes thus far discussed, 11 other azo dyes were investigated during this study but the analytical recovery methodology did not produce satisfactory recoveries from the various matrices for these dyes. Table 6 identifies these dyes.

Conclusions

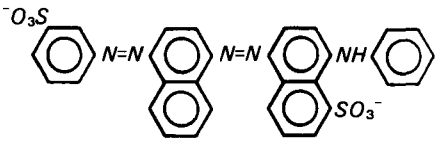
1. A total of 18 water soluble azo dyes were successfully monitored in wastewater and sludge samples collected from pilot-scale ASP treatment systems. The study of 11 additional dyes was attempted but could not be accomplished because of poor analytical recovery from wastewater and/or sludge samples.
2. Based on the compounds tested in this study, high water solubility, as judged by the degree of sulfonation, seemed to be a major factor in preventing an azo dye compound from being either apparently adsorbed or biodegraded by the ASP.
3. Of the 18 dyes studied, 11 compounds were found to pass through the ASP substantially untreated, 4 were significantly adsorbed onto the WAS and 3 were apparently biodegraded.

Recommendations

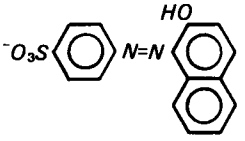
1. Since several azo dyes passed through the ASP relatively untreated, further investigations into how to remove these compounds, and others like them, may be necessary
2. Investigations into the degradation products resulting from the aerobic biodegradation of azo dyes may be necessary to determine if the degradation products, such as aromatic amines, persist in the water
3. For those compounds that strongly adsorb onto WAS, investigations into their fate in anaerobic environments (e.g., anaerobic digesters or landfills) would be of value.
4. Additional testing of structurally related compounds to those tested in this study may allow structure activity relationships to be developed.



C.I. Acid Black 1



C.I. Acid Blue 113



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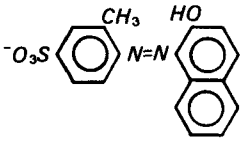

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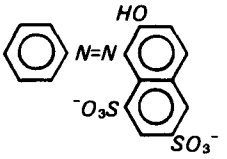
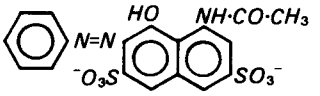
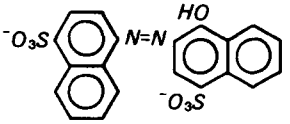


C.I. Acid Orange 10



C.I. Acid Red 1



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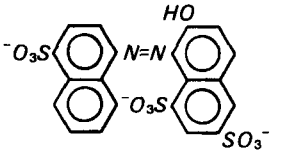
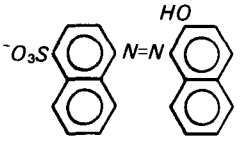
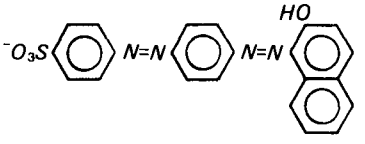


C.I. Acid Red 18

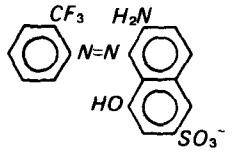


C I Acid Red 88

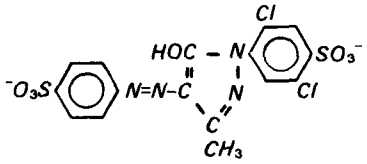


C.I. Acid Red 151

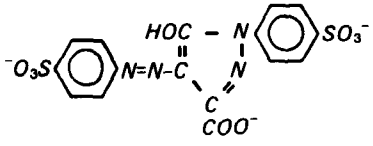
Figure 1. *Chemical structures of test dyes.*



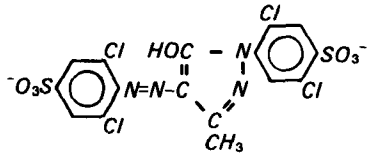
C.I. Acid Red 337



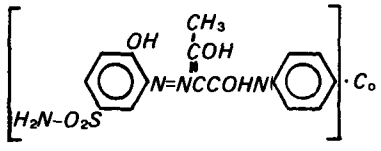
C.I. Acid Yellow 17



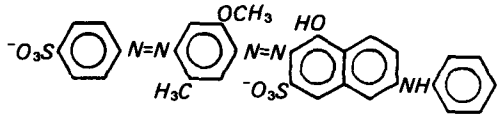
C.I. Acid Yellow 23



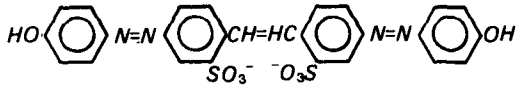
C.I. Acid Yellow 49



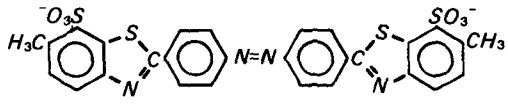
C.I. Acid Yellow 151



C.I. Direct Violet 9



C.I. Direct Yellow 4



C.I. Direct Yellow 28

Figure 1. *(continued).*

Table 3. *Percent Recovery of Test Dyes from Sample Matrices*

	Sample Matrix					
	Org. Free Water		Wastewater		Mixed Liquor	
Dye Compound Name	1 mg/L	5 mg/L	1 mg/L	5 mg/L	1 mg/L	5 mg/L
C.I. Acid Black 1, Run 1	90	93	87	72	87	80
C.I. Acid Black 1, Run 2	69	78	79	90	77	83
C.I. Acid Blue 113	61	89	95	88	85	76
C.I. Acid Orange 7, Runs 1 and 2	101	97	97	97	98	90
C.I. Acid Orange 8	115	111	103	101	86	94
C.I. Acid Orange 10	**	**	91	80	74	73
C.I. Acid Red 1	**	**	105	95	65	92
C.I. Acid Red 14	101	90	98	97	88	92
C.I. Acid Red 18	98	98	94	88	87	90
C.I. Acid Red 88	93	106	92	82	109	96
C.I. Acid Red 151, Run 1	74	85	91	83	94	90
C.I. Acid Red 151, Run 2	**	**	103	83	101	84
C.I. Acid Red 337	89	88	95	88	95	85
C.I. Acid Yellow 17	101	98	92	97	99	97
C.I. Acid Yellow 23	108	102	103	103	102	103
C.I. Acid Yellow 49	90	93	99	100	99	99
C.I. Acid Yellow 151	**	**	116	98	109	113
C.I. Direct Violet 9, Run 1	**	**	120	92	87	89
C.I. Direct Violet 9, Run 2	**	**	103	98	101	95
C.I. Direct Yellow 4	86	90	81	92	93	94
C.I. Direct Yellow 28	96	98	94	87	101	97

**not tested.

Table 4. *Dye Concentrations Data Summary*

	Low Spike +					High Spike +				
Dye Compound Name	INF	PE	ASE	SOL	RES*	INF	PE	ASE	SOL	RES*
Acid Black 1, Run 1	0.53	0.44	0.41	0.40	0.13	2.21	2.20	2.29	2.07	0.47
Acid Black 1, Run 2	0.43	<0.11	0.28	0.19	<0.04	2.59	<0.75	2.02	1.41	0.13
Acid Blue 113	1.00	0.84	0.07	0.04	3.98	5.27	4.55	0.84	0.44	19.86
Acid Orange 7, Run 1	0.99	0.95	0.19	<0.08	<0.03	4.96	5.34	0.24	0.15	<0.03
Acid Orange 7, Run 2	1.12	1.04	0.31	0.25	<0.03	6.18	5.53	0.77	0.58	<0.03
Acid Orange 8	0.80	0.82	<0.03	<0.02	<0.03	4.39	4.02	<0.10	<0.04	<0 03
Acid Orange 10	1.17	0.96	1 01	0.88	<0.03	5.44	5.55	5.49	4.94	<0.04
Acid Red 1	1.01	0.90	0.89	0.83	<0.01	4.71	4.43	4.48	4.43	0.05
Acid Red 14	0.90	0.66	0.77	0.74	<0.03	4.61	2.81	4.16	3.98	<0 11
Acid Red 18	1.21	1.23	1.33	1.12	<0.05	5.11	4.54	4.71	4.76	<0.04
Acid Red 88	**	0.68	0.04	0.02	0.11	**	3.96	<0.01	<0.01	0.07
Acid Red 151, Run 1	**	0.56	0.17	0.08	2.90	**	3.64	0.44	0.17	19.86
Acid Red 151, Run 2	0.96	0.71	0.24	0.09	2.61	4.81	4.31	0.54	0.36	18.85
Acid Red 337	1.20	1.13	0.93	0.68	1.15	5.46	5.06	4.40	3.61	4.08
Acid Yellow 17	0.97	0.95	0.92	0.93	<0.06	4.58	4.51	4.55	4.45	<0.05
Acid Yellow 23	1.33	1.23	1.32	1.30	<0.03	5.08	5.25	5.39	5.35	<0.03
Acid Yellow 49	1.14	1.14	0.84	0.86	0.14	5.17	5.42	3.59	3.79	0.44
Acid Yellow 151	1.29	0.61	0.49	0.26	0.46	6.44	4.05	4.08	2.66	4.31
Direct Violet 9, Run 1	0.95	0.78	0.41	0.21	2.93	5.30	4.71	0.99	0 38	23.38
Direct Violet 9, Run 2	0.98	0.83	0.61	0.32	1.74	5.22	4.72	1.32	0.59	23.44
Direct Yellow 4	0.84	0.76	0.76	0.58	0.08	3.90	3.31	3.17	2.97	0.21
Direct Yellow 28	0.93	0.87	0.18	0.11	5.18	3.74	3.69	0.63	0.25	22.83

+ Concentration in mg/L.
* Mass in mg of dye adsorbed/gm of MLSS.
**Not sampled.

Table 5. Mass Balance Data Summary

	Low Spike		High Spike	
Dye Compound Name	% Recovered	% Adsorbed	% Recovered	% Adsorbed
Acid Black 1, Run 1	96	3	105	2
Acid Black 1, Run 2	244	6	265	2
Acid Blue 113	74	66	65	47
Acid Orange 7, Run 1	19	< 1	4	< 1
Acid Orange 7, Run 2	30	< 1	14	< 1
Acid Orange 8	4	1	2	< 1
Acid Orange 10	104	< 1	98	< 1
Acid Red 1	98	< 1	101	< 1
Acid Red 14	116	1	148	< 1
Acid Red 18	107	< 1	104	< 1
Acid Red 88	7	< 1	< 1	< 1
Acid Red 151, Run 1	73	44	78	66
Acid Red 151, Run 2	82	50	70	58
Acid Red 337	95	14	94	9
Acid Yellow 17	98	1	101	< 1
Acid Yellow 23	107	< 1	103	< 1
Acid Yellow 49	75	1	68	1
Acid Yellow 151	89	13	114	17
Direct Violet 9, Run 1	93	43	89	69
Direct Violet 9, Run 2	100	29	92	66
Direct Yellow 4	99	1	96	1
Direct Yellow 28	78	59	75	59

Table 6. *Dye Compounds Not Tested Due to Poor Recovery*

Colour Index Name	Colour Index Number
C.I. Acid Blue 92	13390
C.I. Acid Blue 158	14880
C.I. Acid Brown 14	14720
C.I. Acid Red 114	23635
C. I. Direct Black 80	31600
C. I. Direct Blue 15	24400
C. I. Direct Blue 78	34200
C. I. Direct Blue 80	24315
C. I. Direct Red 24	29185
C. I. Direct Red 80	35780
C. I. Direct Red 81	28160

Glenn M. Shaul, Clyde R. Dempsey, *and* ***Kenneth A. Dostal*** *are with the Water Engineering Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH 45268.*

The complete report, entitled "Fate of Water Soluble Azo Dyes in the Activated Sludge Process," (Order No. PB 88-208 251; Cost: $14.95, subject to change) will be available only from:

National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone: 703-487-4650

The EPA authors can be contacted at:

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PERMIT No. G-35

Official Business
Penalty for Private Use $300

EPA/600/S2-88/030