United States
Environmental Protection
Agency

Risk Reduction
Engineering Laboratory
Cincinnati, OH 45268

Research and Development

EPA/600/S2-89/051 Jan. 1990

EPA

Project Summary

Aerobic and Anaerobic Treatment of C. I. Disperse Blue 79

David A. Gardner, Thomas J. Holdsworth, Glenn M. Shaul , Kenneth A. Dostal, and L. Don Betowski

This study summarized here was conducted to determine the fate of C.I. Disperse Blue 79, one of the largest production-volume dyes, and select biodegradation products in a conventionally operated activated sludge process and in an anaerobic sludge digestion system. To achieve this objective, a pilot study was conducted from November 1987 to February 1989. Two continuous-feed pilot-scale wastewater treatment systems, one control and one experimental, were operated during the pilot study. The experimental treatment system was fed screened, raw municipal wastewater dosed with a target concentration of 5 mg/L of active ingredient in the commercial formulation of C. I. Disperse Blue 79. The control system was fed only the screened, raw municipal wastewater. After acclimation and after steady state conditions were reached, samples from each system were analyzed for the dye and related compounds. A bench-scale activated sludge system was also operated to assess the fate of dye degradation products from the anaerobic digester in an aerobic treatment system. This system was operated to simulate the recycle of digester supernatant to the head-end of a typical wastewater treatment system. The results of this extensive research project are presented in Volume I of the full report. Findings are presented regarding: (1) the development of an analytical procedure to determine C.I. Disperse Blue 79 in various sample matrices; (2) the effect of C.I. Disperse Blue 79 on the operation of an activated sludge system and an anaerobic digester; (3) the fate of the dye in the treatment systems; and (4) the detection of any degradation products in the systems. Laboratory and operating data collected during the study are presented in Volume II.

This Project Summary was developed by EPA's Risk Reduction Engineering 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 fate of specific azo dyes in wastewater treatment facilities is unknown since few detailed studies have been reported in the literature. A better understanding of this large class of organic compounds is necessary to assess their effect on the environment and on human health. Several azo dyes and possible biodegradation products, such as aromatic amines, have been shown to be, or are suspected to be, carcinogenic. C.I. Disperse Blue 79, one of the largest production-volume dyes, is a water insoluble bromodinitroaniline-derived compound. The empirical formula for the bromomethoxy form of C.I. Disperse Blue 79 used in this study is $C_{23}H_{25}BrN_6O_{10}$, the molecular weight is 625.4, and the structural formula is shown in Figure 1.

The purpose of this study was to determine the fate of C.I. Disperse Blue

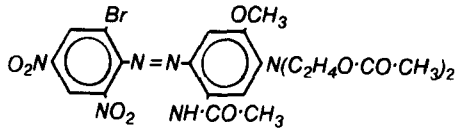

Figure 1. C. I. Disperse Blue 79

79 and select biodegradation products in a conventionally operated activated sludge process and in an anaerobic sludge digestion system. Before testing, an analytical procedure for measuring C.I. Disperse Blue 79 concentrations was developed. Two continuous-feed, pilot-scale wastewater treatment systems (one control [Unit 1] and one experimental [Unit 2]) were operated at the Milwaukee Metropolitan Sewerage District (MMSD) South Shore Wastewater Treatment Plant.

In addition to these pilot-scale systems, a bench-scale activated sludge system (Unit 3) was operated to assess the fate of dye degradation products from a digester in an aerobic treatment system. This system was operated to simulate the recycle of digester supernatant to the head-end of a typical wastewater treatment system.

Experimental Procedures

The two pilot-scale treatment systems were operated for the entire study, from November 1987 to February 1989. The bench-scale system was operated from November 1988 to February 1989.

C.I. Disperse Blue 79 Extraction and Analysis

Because a reliable method for dye analysis was needed to determine the fate of C.I. Disperse Blue 79 in the treatment system, an analytical procedure was developed. Various extraction methods and solvents were investigated to develop a suitable extraction procedure to prepare samples for high-performance liquid chromatography (HPLC) analysis.

Pilot-Scale Treatment Systems

Both pilot-scale activated sludge systems included a contact tank, a conical-shaped primary clarifier, an aeration basin, and a conical-shaped secondary clarifier. The contact tanks were installed to ensure the dye was mixed with the feed and to obtain a 30-min contact time between the raw wastewater and the dye. The primary and secondary clarifiers were approximately 49L, and the aeration tanks were approximately 185 L.

The activated sludge basins were separated into three cells to operate as a plug-flow system. Peristaltic pumps supplied the screened, raw wastewater to the contact tanks. Gravity moved the wastewater from the contact tanks to the primary clarifiers, then to the aeration basins, and on to the secondary clarifiers. Activated sludge was wasted from the aeration basins via peristaltic pumps. Primary sludge was wasted manually once each day. The target hydraulic retention time (HRT) was 5.5 hr and the solids retention time (SRT), 7 days.

The anaerobic digesters were cylindrical-shaped vessels constructed of clear PVC. Each digester had a total volume of 70 L with an operating volume of 39 L. The digesters were completely mixed and heated to maintain an operating temperature of 35°C. Gas production from the digesters was monitored with gas meters.

Waste activated sludge and primary sludge from each activated sludge unit were mixed, thickened, and fed to the respective anaerobic digesters. The target SRT of the anaerobic digesters was 15 days and the target loading was 1.2 kg total volatile solids (TVS)/m^3•day.

The experimental treatment received screened, raw wastewater dosed with a target concentration of 5 mg/L of the active ingredient in C.I. Disperse Blue 79. The control system received only the screened, raw wastewater. After acclimation and steady state conditions were reached, the following samples from each system were analyzed for the dye and related compounds: influent, primary effluent, activated sludge effluent, primary sludge, waste activated sludge, digester feed, digester supernatant, and digester effluent.

Bench-Scale Treatment System

The bench-scale system was an activated sludge unit operated on a feed mixture prepared from the experimental system. The activated sludge unit consisted of a 6-L conical reactor, which served as the aeration basin; a 2-L inner cone for solids recycle; and a 125-ml clarifier tube for effluent clarification. Peristaltic pumps were used to deliver the feed and remove waste activated sludge from the unit.

The feed mixture included primary effluent from the experimental system, supernatant from digester
preparation (primary and waste acti
sludge thickening), and centrate
centrifuging digested sludge from
anaerobic digester. The mixture
prepared to simulate the recycl
digester supernatant and primary
thickened waste activated slu
supernatant to the head-end
treatment plant.

Results and Discussion

C.I. Disperse Blue 79 Extracti and Analysis

Initial experiments with two lic
phase extractions did not yield
acceptable procedure for C.I. Disp
Blue 79; therefore, the approach
changed to dispersing the aqu
sample in acetonitrile. A spectrop
meter monitored the extrac
procedure. C.I. Disperse Blue 79 con
trations in the extracts were anal
with the use of HPLC.

Analytical spike and analyt
duplicate analyses (part of the Qu
Assurance/Quality Control (QA/
program) monitored the accuracy
precision of the extraction procedure
HPLC analyses. For the 57 analy
duplicate analyses performed
spectrophotometry, the average rel
difference was 4.3% with a stan
deviation of 6.7%. For the 29 analy
spike analyses performed
spectrophotometry, the average s
recovery was 105% with a stan
deviation of 13.8%. For the se
analytical duplicate measurem
performed on the HPLC, the ave
relative difference was 6.8% wi
standard deviation of 9.1%. Th
replicate analyses performed on
same sample had an average rel
difference of 4.8% and standard devi
of 3.1%.

Field spike samples prepared
field duplicate samples ensured
proper sample collection proced
were used. The average rela
difference for HPLC analyses was 1
with a standard deviation of 15.3%.

Pilot-Scale Systems Operatio

Operating data were colle
throughout the entire period the syst
were operated. Because of analy
capacity limitations, however, the
scale activated sludge systems,
anaerobic digesters, and the bench-s
activated sludge system were
sampled over the entire period for
analyses. Thus, all discussion

quipment performance include only data uring the time dye analyses were nducted.

Operating and analytical data for the lot-scale activated sludge units are mmarized in Table 1. The data for both nits 1 and 2 were similar. The average COD value for Unit 2 was 73.5 mg/L and t for Unit 1 was 59.2 mg/L. Although e slightly higher effluent TCOD value Unit 2 may have been caused by ding dye to the unit, the data indicate t the overall performance of the perimental activated sludge system s not affected by this addition.

ble 1. Summary of the Pilot-Scale Activated Sludge Systems' Operational and Analytical Data for the Dye Testing Period

Parameter	Unit 1	Unit 2
ed data		
TSS (mg/L	*238*	*211*
TCOD (mg/L)	*364*	*375*
TBOD (mg/L)	*182*	*177*
NH_3-N (mg/L)	*22.5*	*20.7*
perational data		
HRT (hr)	*5.28*	*5.28*
SRT (days)	*5.94*	*5.87*
ixed liquor data		
Temperature (°C)	*20.0*	*20.0*
pH (range)	*6.8-8.0*	*6.8-7.6*
DO, Cell 1 (mg/L)	*2.4*	*2.8*
DO, Cell 2 (mg/L)	*3.6*	*3.5*
DO, Cell 3 (mg/L)	*3.6*	*3.9*
TSS (mg/L)	*3,030*	*3,060*
0_2 Uptake rate (mg/L hr)	*6.8-8.0* 73.1	*6.8-7.6* 58.0
SSVI (ml/g)		
rimary effluent data		
TSS (mg/L)	*134*	*139*
NH_3-N (mg/L)	*22.7*	*21.7*
nal effluent data		
TCOD (mg/L)	*59.2*	*73.5*
TBOD (mg/L)	*16*	*21*
TSS (mg/L)	*27*	*31*
NH_3-N (mg/L)	*0.26*	*0.18*

The anaerobic digester's operating id analytical data are summarized in ible 2. The feed, effluent, and erational data indicate no significant ference between the two units. No verse affect was detected on the eration of the experimental digester as result of adding dye.

ye and Related Compounds esults

The influent and effluent streams (feed ream, primary clarifier effluent, primary sludge, waste activated sludge, and final clarifier effluent) from the experimental activated sludge systems were sampled and analyzed for C.I. Disperse Blue 79 and any related compounds to determine the fate of the dye in the treatment system. The average dye and TSS concentrations from the Unit 2 samples are summarized in Table 3. Influent and waste mixed liquor samples were analyzed from Unit 1. The dye was not detected in any of the control unit samples analyzed (i.e., no background concentration of dye was present in the raw municipal wastewater feed).

Table 2. Summary of the Anaerobic Digesters' Operational and Analytical Data for the Dye Testing Period

Parameter	Unit 1	Unit 2
Feed data		
TSS (mg/L)	*21,300*	*21,400*
TS (%)	*2.42*	*2.42*
TVS (%)	*1.81*	*1.82*
Effluent data		
pH (range)	*6.6-7.0*	*6.6-7.0*
Temperature (°C)	*35.0*	*35.0*
TSS (mg/L)	*12,700*	*12,200*
TS (%)	*1.46*	*1.48*
TVS (%)	*0.94*	*0.97*
Operational data		
Alkalinity (mg/L)	*2,930*	*2,820*
Volatile acids (mg/L)	*< 51*	*< 50*
Loading (kg TVS/m^3 day)	*1.21*	*1.22*
TVS reduction (%)	*47.8*	*46.4*
Gas production (m^3/kg TVS destroyed)	*0.76*	*0.87*
Percent CH_4 in gas	*58.9*	*57.9*

The average dye concentration in the Unit 2 feed to the primary clarifier was 4.40 mg/L and the average final effluent concentration was < 0.93 mg/L, so that the average dye removal was greater than 79%. Although 5 of 19 analyzed effluent samples were below the 0.25 mg/L detection limit, the effluent dye concentration varied from < 0.25 mg/L to 3.70 mg/L. The variation in effluent dye concentration may have been caused by the variation in effluent TSS concentration.

The correlation coefficient between TSS and dye concentrations in the Unit 2 effluent was determined to be 0.78. In addition, calculations performed on Table 3 data show that each gram of suspended solids in the waste activated sludge contained 30 mg of dye whereas each gram of suspended solids in the final effluent contained 33 mg of dye. These data indicate that the dye has a high affinity for the activated sludge solids. Approximately 21% of the dye fed to the unit was in the final effluent; however, most of the dye was probably in the suspended solids in the effluent. The average final effluent TSS concentration was 28 mg/L. Lowering this TSS concentration by improving solids removal in the final clarifier could result in a lower dye concentration in the final effluent.

Table 3. Average C. I. Disperse Blue 79 and TSS Concentration in the Unit 2 Experimental Activated Sludge Unit Samples

Sample Location	C.I. Disperse Blue 79 (mg/L)	TSS (mg/L)
Feed	*4.40*	*212*
Primary effluent	*4.71*	*133*
Primary sludge	*31.8*	*14,500*
Waste activated sludge	*93.5*	*3,060*
Final effluent	*< 0.93*	*28*

Mass balance calculations were performed with the use of the measured dye concentrations and measured flowrates for each process stream. Mass balance calculations across the entire activated sludge system showed that an average of 86.5% of the dye contained in the feed stream was accounted for in the effluent streams. The primary sludge contained an average of 3.6% of the dye fed to the system; waste activated sludge, 62.3%; and final effluents, 20.4% (the percentages of the three streams do not equal 86.5% because of rounding off the individual values). Since most of the dye fed to the system was recovered and no other related compounds were detected, it can be concluded that no significant biodegradation of C.I. Disperse Blue 79 occurred in the activated sludge system.

Feed sludge and effluent (digested sludge) samples from both the control and experimental digesters were analyzed for dye content. Detectable concentrations of dye were identified by HPLC-UV in 5 of 10 control-unit feed samples and in 4 of 10 effluent samples. The average concentrations were low, however, at < 1.45 mg/L for the feed samples and < 1.22 mg/L for the effluent

sample. The low level of dye in these control unit samples is negligible when compared with the much higher concentrations of dye in the experimental unit samples.

The average experimental unit feed dye concentration was 443 mg/L, and the average effluent concentration was 7.86 mg/L. On the average, 98.2% of the dye contained in the feed sludge was degraded in the anaerobic digester.

Thermospray ionization mass spectrometry was used to identify degradation products of C.I. Disperse Blue 79 in the anaerobic digester effluent. With this ionization technique, the parent dye was observable, but because of the electronegativity of many of the functional groups on the molecule (e.g., NO_2, Br), the sensitivity of the technique for this compound was poor. Four major degradation compounds were tentatively identified and found in significant amounts in the digester effluent. Their exact identity and amounts have not been verified because appropriate analytical standards were not available. The degradation products showed a better response than the parent dye by the ionization technique; the increased sensitivity probably resulted from either removal or reduction of the electronegativity that was suppressing the sensitivity of C.I. Disperse Blue 79. The relative amounts of these compounds (A, B, C, and D having molecular weights of 283, 358, 400, and 478 daltons, respectively) measured in one set of samples are summarized in Table 4. The relative amounts of these compounds were greater in the digester effluent than in the digester feed. (Information about the structure of these compounds is discussed in detail in Volume I of the full report).

Some of the potential degradation pathways of C.I. Disperse Blue 79 could liberate bromide from the dye compound. Anaerobic digester feeds and effluents were analyzed to determine if bromide was liberated in the digester. In one set of data from the control unit, sample analyses did not show any significant difference in bromide concentration between the feed and effluent (Table 5). In both sets of data from the experimental unit, however, the bromide concentration was much lower in the feed than in the effluent. The two feed samples averaged 2.50 mg/L, and the two effluents averaged 39.0 mg/L. For the one set of samples for which dye analyses are also available, the dye concentration was reduced from 250 mg/L to 9.46 mg/L. These data indicate that bromide was being liberated during dye degradation in the anaerobic digester.

Bench-Scale Activated Sludge System

During normal operation of a wastewater treatment system, the supernatant from sludge lagoons or other digester sludge thickening operations is returned to the head-end of the plant for treatment. The bench-scale activated sludge system (Unit 3) was operated to study the fate of dye degradation products from the anaerobic digester in an activated sludge system. The supernatant from the sludge thickening operation used to prepare the digester feed was mixed with centrate from centrifuging digester effluent and primary effluent to prepare feed for Unit 3. The supernatant was added to simulate the effluents produced from thickening waste activated sludge in a typical plant.

The operating and analytical data from Unit 3 are presented in Table 6. The average HRT was 6.04 days, which was slightly higher than the Unit 2 value of 5.28 days; the average SRT for Unit 3 was 4.83 days, which was lower than the Unit 2 average of 5.87 days. The Unit 3 average SRT was lower than the target value of 7 days because of a relatively high average effluent TSS value of 40 mg/L. The bench-scale unit settling performance was more variable than that in the pilot units.

The average effluent TCOD and TBOD values were also higher than the pilot unit values. The higher effluent values probably resulted from the higher TSS levels in the final effluent. The performance of Unit 3 with respect to TSS, TBOD, and TCOD removal was not as good as that of the pilot units but was typical of a bench-scale unit.

Table 7 summarizes the dye data from the bench-scale unit feed, waste activated sludge, and final effluent sample analyses. The average feed dye concentration was 3.43 mg/L and the average effluent concentration was 1.32 mg/L, for a removal efficiency of 62%. The effluent concentration was probably high because of the relatively high TSS concentration in the final effluent.

Mass balance calculations of the dye across Unit 3 showed that an average of 75.3% of the dye fed to the unit was accounted for in the effluents from the unit. The mass balance for Unit 2 showed 86.5% of the dye was recovered. Although the recovery from Unit 3 was slightly lower, it does not appear that significant degradation of the d occurred in the bench-scale activa sludge system.

Degradation products of C.I. Dispe Blue 79 were also monitored in influent, effluent, and waste sludge fr Unit 3. Because no positive identificat was made of the by-products, quan cation was not possible. Some gen observations can, however, be ma concerning the degradation produ based on relative amounts. The obser trend indicated that the concentration these compounds decreased across U 3. The final effluent samples alwa contained the lowest concentrations the degradation products, but because limited data, further conclusions can be drawn. Further evaluation of degradation products and their fate biological treatment systems may subject for further project work.

Conclusions

1. The addition of C.I. Disperse Blue did not adversely affect the opera of the pilot activated sludge unit that of the anaerobic digester. B the control and experimer activated sludge units produ effluents typical of munici wastewater treatment systems. anaerobic digesters achieved vol solids reductions within the nor operating range for munici digesters.
2. No evidence of C.I. Disperse Blue degradation in the activated slu systems was found. Mass bala calculations showed that, on avera 86.5% of the dye contained in feed to the system was presen the effluent streams.
3. The majority of the C.I. Dispe Blue 79 fed to the activated slu system was removed in the wa activated sludge. The average mass balance obtained around system was 86.5%; the dye partitioned in the effluent streams follows: 3.6% in the primary slud 62.3% in the waste activated slud and 20.4% in the final effluent.
4. The C.I. Disperse Blue 79 degraded in the anaerobic dige The dye concentration was redu from an average feed value of mg/L to an average effluent valu 15.0 mg/L, or a 97.4% reduction.
5. Possible degradation products of dye were detected in the dige effluent. Although some prelimi measurements were made to ide the structure of these compounds

Table 4. *Mass Spectrometric Analysis of Anaerobic Digester Samples*

Sample Location	Relative Sample Amounts (Arbitrary Units)			
	A(MW 283)	B(MW 358)	C(MW 400)	D(MW 478)
Control digester feed	26,000	7,300	7,400	1,700
Experimental digester feed	24,000	6,400	6,300	43,000
Experimental digester effluent	568,000	264,000	365,000	225,000

Table 5. *Bromide Analyses of Anaerobic Digester Samples*

Sample Location	Sample Date	Bromide Concentration (mg/L)	C.I. Disperse Blue 79 Concentration (mg/L)
Experimental unit feed	1/5/89	1.75	*
Experimental unit effluent	1/5/89	40.8	*
Control unit feed	2/7/89	0.85	*
Control unit effluent	2/7/89	0.66	*
Experimental unit feed	2/7/89	3.24	250
Experimental unit effluent	2/7/89	37.1	9.46

*Analysis not performed.

Table 6. *Summary of Activated Sludge Unit 3's Operational and Analytical Data*

Parameter	Average Value
Feed	
TSS (mg/L)	130
TCOD (mg/L)	336
TBOD (mg/L)	158
Operation data	
HRT (hr)	6.04
SRT (days)	4.83
Mixed liquor data	
Temperature (°C)	21.5
pH (range)	6.8-8.1
DO (mg/L)	5.6
TSS (mg/L)	1,650
Final effluent data	
TSS (mg/L)	40
TCOD (mg/L)	116
TBOD (mg/L)	31

Table 7. *Bench-Scale Activated Sludge System's C. I. Disperse Blue 79 Analytical Results*

Sample Location	C. I. Disperse Blue 79 (mg/L)	TSS (mg/L)
Feed	3.43	14.5
Waste activated sludge	37.6	2,150
Final effluent	1.32	53

positive identification or quantification of the compounds was made.

6. Based on limited semi-quantitative results, some of the dye degradation products from the anaerobic digester were destroyed when treated in a bench-scale activated sludge system.

The full report was submitted i fulfillment of Contract No. 68-03-3371 I Radian Corporation under th sponsorship of the U.S. Environment Protection Agency.