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Biological Removal of Colour from Textile Finishing Washwater

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ABSTRACT

Most dyes are by nature recalcitrant. An initial dye biodegradation study indicates that given the right process conditions, colour due to azo dyes can be removed biologically from wastewater. Biodegradation has been found to proceed via cleavage of azo bond(s). Anoxic condition was found to be necessary for biological colour removal. The removal rate was faster in the presence of a readily biodegradable co-substrate.

ABSTRAK

Kebanyakan pewarna secara semulajadi adalah stabil. Kajian pembiorosotan pewarna menunjukkan bahawa warna dari kandungan pewarna azo dalam air buangan dapat dihapuskan secara biologi jika keadaan proses yang sesuai digunakan. Pembiorosotan didapati melibatkan pemutusan ikatan azo. Pembiorosotan ini didapati memerlukan keadaan anoksik. Kadar pengurangan warna didapati lebih cepat jika bahan mudah membiorosot dibekalkan bersama.

INTRODUCTION

The textile industry is a well known source of pollution in many countries including Malaysia, where it ranks third as contributor of BOD and fifth in volume of the total industrial wastewater (Maheswaran et al., 1980). Problems of textile industries wastewaters (WW) are due to its colour, pH and organics content. Most of this wastewater comes from textile finishing processes (Tsang, 1982) which consist of cleaning and modification of cloth (such as scouring, desizing, bleaching and mercerising), dyeing and application of special finishes such as water and flame proofing. As up to 90% of textile finishing WW (TFWW) may come from the dyeing stages (Kertell and Hill, 1982), TFWW is generally strongly coloured (Shelley et al., 1976; Netzer and Beszedits, 1975; Junkins, 1982). From the organics and colour contents of a few TFWW (Table 1), it can be seen that TFWW treatment has to remove about 90 – 97% BOD, 60 – 97% COD and up to 99% colour in order to meet the U.S. EPA standards.

The treatment of (TFWW) may be via physicochemical or biophysicochemical processes. An example of the former is cooling and equalization, followed by coagulation, settling and pH adjustment of treated effluent (Kertell and Hill, 1982). Biophysicochemical processes commonly used are colour removal via

chemical coagulation or activated carbon absorption followed by an activated sludge type of process. For removal of organics, biological processes are cheaper than purely chemical processes (Rovel, 1978; Parish, 1977) since fewer chemicals are needed and sludge disposal costs are lower. However, due to the recalcitrance of dyes in the activated sludge type process, physical or chemical colour removal process is generally used. Although several studies have shown textile dyes to be recalcitrant (Ghosh et al., 1978; Porter and Snider, 1976; Yang and Pescod, 1977), many recalcitrant organics have been biodegraded after acclimation. Acclimation exposes biomass to increasing concentrations of recal-

TABLE 1. Organics content and colour of textile finishing wastewater

Organics mg/l		Colour	Reference
BOD	COD	Units	
200	545	640 APHA	Shriver & Dague, 1977
180	1300	350 APHA	Ghosh et al., 1978
634	*	11300 APHA	Davies et al., 1977
371	*	113 APHA	Kertell & Hill, 1982
550	850	325 ADMI	Kemmer & McCallion, 1979
150 – 600	360 – 1400	*	Tsang, 1982
535	3720	*	Menon, 1978
437	500	*	Noguchi et al., 1974
500	1300	*	Rovel, 1978
*	1260	high	Shelley et al., 1976
[27]	[133]	*	Netzer & Beszedits, 1975
20	*	(100 APHA)	Kertell & Hill, 1982

Note:
* Not Available
[ ] U.S. EPA discharge effluent standards as of 1975.
( ) U.S. EPA discharge effluent standards as of 1982.
Magnitude of APHA and ADMI units are similar (Davies et al. 1977).

citrant organics, thus allowing microorganisms to adapt to these organics. A study by Weeter and Hodgson (1977) using six dyes, each at 3 — 21 mg/ℓ showed that microorganisms can be acclimated to dyes and better colour removal can be achieved by using acclimated biomass. This paper reports the findings from an on-going study on biodegradation of several azo dyes.

MATERIALS AND METHODS

ACCLIMATION

Mixed cultures from sewage (Pantai Dalam Sewage Treatment Plant, Kuala Lumpur) and garden soil microorganisms were acclimated to five dyes (Table 4), with one dye per culture. The azo dyes used in this study were supplied by Hoechst AG, Frankfurt. Each 250 conical flask containing 200 ml culture and 5 mg/ℓ were maintained as semi-continuous activated sludge process (i.e., with continuous aeration but once a day feeding and effluent removal) operating at hydraulic residence time of 2 days (τ = 2d) and without wasting biomass. 100 ml new feed was added each day to the 100 ml settled culture retained after settling and effluent removal. The feed consisted of salt medium (composition in Table 2) buffered with pH 7.5 phosphate buffer (to maintain the flask content at pH 7.0 — 7.2) and a dye. All studies were carried out at room temperature (25 — 27 C). Absorbance measurements at each dye's λ max (Table 4) using Spectrophotometer Spectronic 21 UVD (Bausch and Lomb) were regularly carried out on the effluent filtrates. Filtration was carried out using GF/C glass fibre filter (Whatman).

TABLE 2. Composition of nutrient salts.

Salt	Concentration, mg/ℓ
$(NH_4)_2SO_4$	500
$MgSO_4 7H_20$	100
$MnSO_4 H_20$	10
$FeCl_3$ $6H_20$	0.5
$CaCl_2$	7.5
1M phosphate buffer PH 7.5	10ml

After a month's run without finding colour removal, another five acclimation flasks were started using the same original seed (which was maintained in the laboratory on salt medium and 1000 mg/ℓ glucose). This group had glucose, at a concentration (C_G) of 1000 mg/ℓ, as co-substrate in its feed. It was maintained at τ = 2d and cell residence time, θ , of 20 days. θ was maintained as such by alternate day wasting of 20 ml mixed culture. After getting a steady absorbance reduction, the feed dye concentration, C_D, was increased to 8 mg/ℓ. It was subsequently increased in increments of 2 mg/ℓ after about a month at the new C_D or when a steady absorbance decrease had been obtained.

BIODEGRADATION CONDITION

The acclimation flasks containing glucose as co-substrate were observed to give greater colour removal at slower aeration rate. This could be due to the degraders being sensitive to shear or able to attack the dye only under anoxic condition. Several batch studies using dye Black B were carried out to determine the process condition that would yield fastest colour removal. The process conditions tested were as in Table 3. The biomass used was generated by maintaining the wastings (from the acclimation to Black B flask) in semi-continuous process with V = 21, τ = 2d, θ = 30d, C_D = 10 mg/ℓ and C_G = 1000 mg/ℓ. All aerated contents were completely mixed by aeration. Non-aerated contents were completey mixed by shaking (Stuart Flask Shaker, Stuart Co. Ltd., UK).

TABLE 3. Biodegration condition

Process Condition	Variables Tested			
	Mixing	Aeration	Glucose	Volatilation
1. Air, C_G = 0		x	x	
2. No air, mixing, C_G = 0		x	x	
3. Air, C_G = 1000 mg/ℓ		x	x	
4. No air, mixing, C_G = 1000 mg/ℓ	x	x	x	
5. No air, no mixing, C_G = 1000 mg/ℓ	x		x	
6. Air, no biomass				x

ANALYSIS OF BIODEGRADATION PRODUCTS

Filtrates of biologically decolourised solution containing Black B were analysed using chloramine-T and thin layer chromatography (TLC). Black B was also chemically reduced using zinc dust and ammonia (Gasparic, 1977) and the resulting products were chromatographed in parallel with biodegradation products on Silica Gel 60 plates (Art. 5721, Merck).

RESULTS AND DISCUSSION

ACCLIMATION

The acclimation group with dye as sole substrate did not show absorbance decrease even after two months of exposure to the dyes. This shows that the dyes are not easily broken by microorganisms, as similarly found by other researchers (Ghosh et al., 1978; Porter and Snider, 1976; Yang and Pescod, 1977). This could not have been due to the nonavailability of suitable microorganism or too low a C_D to induce response since in the acclimation group with glucose as co-substrate absorbance reduction occurred at least for several of the dyes (Table 4). Removal due to absorption onto biomass may be negligible as the biomass remained unstained. Hence, presence of glucose has brought about a change in the concentration of the original dye molecule. The role of glucose is suggested later.

Slow aeration rate (about 50 ml/min/flask) was used during acclimation when it was noticed that this effected a greater absorbance decrease. With this acclimation condition, the feed and effluent concentrations reached after about 16 months are given in Table 4. The absorbance decrease can be attributed to both acclimation of biomass and the slow aeration rate used. The former can be said to occur as at each C_D increase, effluent C_D

TABLE 4. Dye concentrations in acclimation

Dye	λ max, nm	Feed C_D, mg/1ℓ	Effluent C_D, mg/ℓ
Red B	506	80	10
Orange 3R	494	28	14
Yellow GL	400	10	10
Brown 3G	475	18	14
Black B	596	64	16

would be higher than the C_D it would settle down to after about a week. At low aeration rate the flocs were fairly large (about 1 mm) and therefore may have inner anoxic regions. Requirement of slow aeration to effect colour removal is explained later.

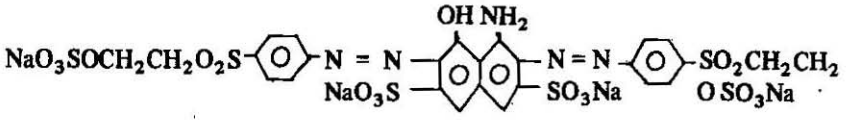
Table 4 shows that some dyes are more recalcitrant than others, the most recalcitrant in the group being Yellow GL. Dye Red B gave highest colour removal.

BIODEGRADATION CONDITION

The rates of abosrbance decrease in Fig. 1 (for the conditions given in Table 3) clearly show that anoxic condition (conditions 2, 5 and 4) favours colour removal. This would explain the effect of glucose mentioned above. Presence of glucose renders the condition more anoxic as oxygen is used up as electron receiver in the metabolism of glucose. Under completely mixed aeration condition, Figure 1 shows that dye remains as a very stable molecule. Mixing under non-aerated condition enhanced colour removal rate. Removal rate for the non-mixed condition in Fig. 1 (condition no. 5) was rather high due to mixing at sampling times.

BIODEGRADATION PATHWAY

Chloramine-T tests on Black B effluent yielded the yellow colour characterising the presence of aromatic amines (Mancy and Weber 1971). If the azo bonds in the Black B molecule are broken, aromatic amines would be produced. The molecular structure of Black B is shown below:



Cleavage for both azo bonds would yield the substituted naphthol 2, 7, 8-triamino-1-naphthol-3, 6-disulphonic acid (TANDSA) TANDSA is characterised as being colourless under strongly reducing conditions but will rapidly turn blue when oxidised by air (Gasparic, 1977). When the colourless effluent from the non-aerated bottle in the batch study using Black B was exposed to air, it rapidly turned bluish. This blue colour would disappear after about 24 hours if the bottle is restoppered but would re-appear if the content was again exposed to air. Presence of TANDSA was confirmed by the TLC of the effluent in conjunction with the products from reduction of Black B using zinc dust and ammonia. This reduction yields TANDSA. The chemical reduction products yielded two spots, a blue visible spot for TANDSA and a UV visible spot for the substituted amine

(Table 5). The effluent yielded several spots, one of which had the same R_f, colour and shape as TANDSA, thus showing that TANDSA was present in Black B effluent. In fact from the TLC of effluent samples taken after several days of incubation, it can be seen that TANDSA is the most stable metabolite from biodegradation of Black B. This is not surprising as the naphthol is highly substituted. Many studies on recalcitrant organics have revealed that recalcitrance increases with the degree of substitution (Tabak et al., 1964; Chu and Kirsch, 1972). However, samples from anoxically incubated content reveal a slow decrease in the TANDSA blue colour and therefore a slow decrease in the TANDSA concentration (Figure 2). The spots with R_f 0.5, 0.4 and 0.3 are most

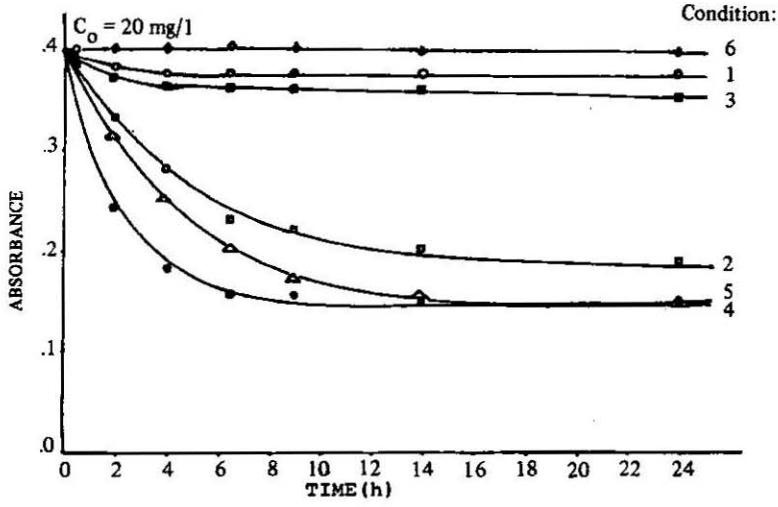


FIGURE 1. Absorbance decrease at different process conditions (for dye Black B)

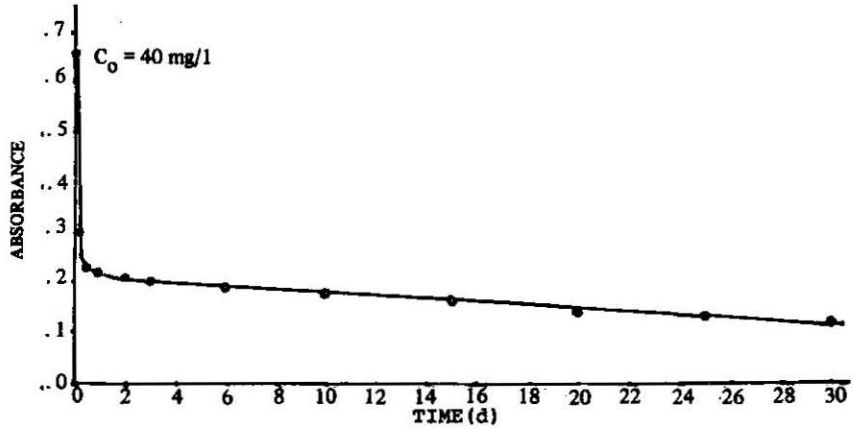


FIGURE 2. Absorbance decrease in batch for dye Black B.

probably due to different substituted amines. Their lower R_f compared to that from chemical degradation shows that biodegradation has increased their polarity and thus reactivity.

TABLE 5. R_f values for Black B
(effluent : n-propanol : ammonia (2:1))

Black B	Chemical reduction Products	Sample 1	Taken 5	After 10	(day): 30	Colour visibility (UV or VIS)
	0.8	0.8	0.8	0.8	0.8	Blue (VIS)
0.7						Bluish Black (VIS)
	0.6					(UV)
		0.5	0.5			(UV)
		0.4	0.4	0.4		(UV)
		0.3	0.3			(UV)

Due to the TLC spots and the conditions required for absorbance reduction to occur it can be said that biodegradation of azo dyes occurs only under anoxic conditions or under conditions where there is an anoxic region (as shown by the acclimation studies). In this condition the azo bond(s) is reduced, i.e., the azo dye acts as hydrogen acceptor. Hence the reduction rate would be enhanced by the presence of a readily metabolised organics, such as glucose, which would act as hydrogen donor. The reduction products are then further broken down, although perhaps more slowly than the cleavage of the azo bond(s), depending on the structure of the metabolite.

The need for anoxic condition means that azo dyes would be very recalcitrant in the activated sludge type of process commonly used for textile finishing wastewater treatment. However, the recurrent interest in anaerobic process for treatment of industrial wastewater augurs well for biological azo dye colour removal from textile finishing wastewater.

CONCLUSION

This study reveals that biodegradation of azo dyes, hence colour removal via biological process, is possible. However, biodegradation occurs only under anoxic condition, thus biological remo-

val is possible only in anaerobic processes or in processes where anoxic regions are present. Under anoxic condition dye biodegradation was found to occur via azo bond(s) cleavage where the dye molecule probably acts as electron acceptor.

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REFERENCES

Chu, J. P. and Kirsch, E. J. 1972. Metabolism of Pentachlorophenol by Axenic Bacterial Culture. *Appl. Microbiol.* 23(5): 1033-1035.

Davies, G. M., Koon, J. H. and Adams, G. E. 1977. Treatment of two Textile Dyehouse Wastewaters. *Proc. 32nd Ind. Waste Conf.* Purdue Uni, 981-997.

Gasparic, J. 1977. Chemical Degraduation Methods. In *The Analytical Chemistry of Synthetic Dyes',* ed. Venkataraman, K. New York: John Wiley & Sons, Inc.

Ghosh, M. M., Woodard, F. E., Sproul, D. J. Knowlton, P. B. and Guertin P. E. 1978. Treatability Studies and Design Considerations for Textile Wastewater. *J. Water Pollut. Control Fed.* 50(8): 1976-1985.

Junkins, R. 1982. Case History: Pretreatment of Textile Wastewater. *Proc. 37th Ind. Waste Conf.* Purdue Uni., 139-146.

Kemmer, F. N. and McCallion, J. 1979. Textile Industry. In *Nalco Water Handbook.* New York: McGraw Hill Book Comp.

Kertell, C. R. and Hill, G. F. 1982. Textile Dyehouse Wastewater Treatment; A case history. *Proc. 37th Ind. Waste Conf.* Purdue Uni., 147-156.

Maheswaran A., Abu Bakar J. and Singam G. 1980. Water Quality Management in Malaysia. Presented at Symposium on the Interdependence of Economic Development and Environmental Quality in South East Asia, Miami Uni., USA, August 5-7.

Mancy, K. H., and Weber, W. J. 1971. *Analysis of Industrial Wastewaters.* New York: Wiley – Interscience.

Netzer, A. and Beszedits, S. 1975. Pollution from Textile Mills: Problems & Solutions. *Water and Pollution Control.*

Noguchi, S., Yamanaka, S. and Suzuki, K. 1974. Treatment of Degumming & Dyeing Wastewater Through Floor Tower Shaped Biofiler Process. *Japan Textile News. No. 232: 91-95*

Parish, G. J. 1977. Textile and Tannery Wastes. In Treatment of Industrial Effluents, ed. Callely, Forster and Stafford. London: Hodder and Stoughton.

Porter, J. J. and Snider, E. H. 1976. Long Term Biodegradability of Textile Chemicals *J. Water Pollut. Control Fed.* 48(9): 2198-2210.

Rovel, J. M. 1978. Treatment of Effluents from Textile Fiber Processing Industries. *Proc. International Conf. On Water Pollution Control in Developing Countries,* Bangkok 21-25 Feb., 493-502.

Shelley, M. M. Randall, C. W. and King, P. H. 1976. Evaluation of Chemical-Biological and Chemical-Physical Treatment for Dyeing and Finishing Waste. *J. Water Pollut. Control Fed.* 48(4): 758-761.

Shriver, L. E. and Dague, R. R. 1977. Textile Dye Process Waste Treatment with Reuse Consideration. *Proc. 42nd. Ind. Waste Conf.,* Purdue Uni., 581-592.

Stracke, R. J. and Baumann, E. R. 1975. Biological Treatment of Toxic Industrial Waste – Performance of an Activated Sludge & Trickling filter Pilot Plant. *Proc. 30th Ind. Waste Conf.,* Purdue Uni., 1131-1160.

Tabak, H. H., Chambers, C. W. and Kabler, P. W. 1964. Microbial Metabolism of Aromatic Compounds I: Decomposition of Phenolic Compound and Hydracarbons by Phenol Adapated Bacteria. *J. Bacteriol.* 87(4): 910-919.

Tsang, W. 1982. Waste Water Treatment. *Textile Asia.*, Oct. 24-38.
Weeter, D. W. & Hodgson, A. G. 1977. Dye Wasterwater Alternatives for Biological Waste Treatment. *Prof. 32nd. Waste Conf.*, Purdue Uni., 1-9.
Yang, P. T. & Pescood, M. B. 1977. Polymerisation and Dyeing Wastewater Characteristics and Their Treatability. *Prof. 32nd Ind. Waste Conf.*, Purdue Uni., 222-241,

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