COLOUR REMOVAL FROM WASTEWATER BY MEANS OF MICROBIAL TREATMENT

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Abstract

Water solutions of different dyes used in the textile industry (Reactive Black 5, Acid Orange 7 and Disperse Yellow 5) were treated under laboratory conditions by means of different microorganisms and/or their metabolites. The following microorganisms were used in this study: *Bacillus megatherium, B.subtilis, B.circulans*, activated sludge from an industrial wastewater treatment plant and a mixed enrichment culture of flocculating bacteria. It was found that the microbial effect towards the dyes was quite specific and the extent of the colour removal varied in a broad range (from 0 to 99 %). These experiments were performed in agitated reactors under batch conditions. Very good results were achieved also by means of permeable reactive barriers inhabited by flocculating bacteria and other metabolically interdependent microorganisms and operating under continuous-flow conditions. The colour removal in these cases varied in the range of about 80-90 % and was similar to that achieved by the well known chemical reagents used in the textile industry.

***Key words:** wastewater, Bacillus megatherium, B.subtilis, B.circulans*

Introduction

The treatment of wastewaters from the textile industry is an important technological problem. In most cases the visible effect from the treatment is the decolourization of the polluted waters [1 - 4]. It must be noted that very often the decolourization is connected with a decrease of the toxicity of the respective wastewaters. This is due to the fact that in some cases the decolourization is a result of the degradation of the dyes present in the polluted waters to non-toxic or, at least, to less toxic products, and in some other cases - of the removal of the dyes from the water phase.

The decolourization of textile dyes has been widely studied and different chemical (oxidation, reduction), physical and physico-chemical (sedimentation, adsorption, ionexchange, membrane filtration) and biological processes have been used to solve this problem. The biological processes are quite varied and involve degradation of the dyes by means of oxidation or reduction of their functional groups, biofloculation and biosorption [5 - 8]. It must be noted that at present the interest towards these biological processes is steadily increasing.

The present paper contains some data about laboratory studies on the microbial decolourization of water solutions of three different dyes which are used in the textile industry.

Materials and Methods

Water solutions of three different dyes used in the textile industry were used in this study (Table 1). The solutions were prepared by dissolving 100 mg of the respective dye into 1000 ml distilled water.

Table 1. Characterization of the dyes used in this study

Commercial name	Producer	Chemical index	Chemical group	Wave length, λ max [nm]
Cibacron Marine	CIBA	Reactive Black 5	disazo	593
-	POLFA Pabianice, Polnad	Acid Orange 7	monoazo	481
Synten Yellow P5G 100 %	BORUTA COLOR Zgierz, Poland	Disperse Yellow 5	azo	392

Various microorganisms were used in the experiments for colour removal of the abovementioned water solutions:

- Different species of the genus *Bacillus* (*B. megatherium, B. subitilis, B. circulans*). These bacteria were grown on a nutrient medium with the following composition: glucose 20 g, peptone 4 g, yeast extract 0.35 g, K_2HPO_4 0.5 g, NH_4Cl 0.02 g, $MgSO_4.7H_2O$ 0.35 g, distilled H_2O 1000 ml. The cultivation was carried out in 500 ml Erlenmeyer flasks containing 180 ml nutrient medium and 20 ml late-log-phase bacterial inoculum. The flasks were agitated on an orbital shaker at 180 rpm and 35°C for 72 hours.

The microbial treatment of the coloured water solutions was performed by two different means: by spent nutrient media containing bacterial cells, secreted bacterial metabolites and residual amounts of nutrients and by washed cell suspensions.

The treatment by the spent nutrient media was carried out in agitated Erlenmeyer flasks containing 30 ml water solution of the respective dye and 30 ml of the spent nutrient medium. The mixing of these two components was carried out for 2 min at 200 rpm, and then for 6 min at 80 rpm. The mixture was then filtered through Whatman paper filter "bleu band" and the colour of the filtrate was measured spectrophotometrically at the respective wave length shown in Table 1, and then was compared with the initial colour of the water solution of the respective dye.

For obtaining bacterial cell suspensions the spent nutrient media were centrifugated at 10 000 rpm for 10 min, the obtained cell precipitates were washed with distilled water and were subjected again to centrifugation. This procedure was repeated to obtain washed cells free of secreted metabolites and nutrients. The precipitated cells were suspended in 30 ml distilled water, the pH of this suspension was adjusted to 3.2 by addition of ice acetic acid. Such washed cell suspensions were mixed with 30 ml of water dye solution and the further treatment follows the procedures described above for the treatment by means of spent nutrient media:

- Microorganisms present in activated sludge from wastewater treatment plant.

The treatment was carried out by means of crude activated sludge containing a varied biocenose as well as by means of enriched mixed culture of flocculating bacteria isolated from the activated sludge. The nutrient medium mentioned above for the growth of *Bacillus spp.* was used for obtaining the enrichment culture. The treatment of the water dye solutions was carried out using the procedures described for the *Bacillus spp.*

The treatment by means of the crude activated sludge was carried out using water suspensions with pH of 3.4 containing ~ 30 wt % activated sludge. 30 ml of such suspensions were mixed with different volumes of water dye suspensions containing 100 mg/l of the respective dye. The mixtures were agitated for 2 min at 200 rpm, and then for 6 min at 80 rpm. The mixtures were then centrifugated for 10 min at 10000 rpm and the supernatants were filtered through Whatman paper filter "bleu band" before the determination of colour intensity.

- Treatment of water solutions of dyes by means of permeable reactive barriers inoculated with activated sludge

The permeable reactive barriers were cylindrical glass columns with a volume of 1600 ml each filled with a mixture consisting of (in wt %): cow manure 75, hay 20, quartz sand 5. Drain zones consisting of gravel lumps (with a particle size of + 15 - 25 mm) and glass cotton were located in the bottom and top zones of the columns. The total water volume of the individual barrier was 880 ml, and its porosity was 68.0 %. The barriers were inoculated by activated sludge containing viable microbial population at density ~ 10^{10} cells/g. The water solutions of dyes were directed to the barriers by means of peristaltic pumps at rates reflecting residence times from 120 to 24 hours. The quality of water was monitored at least once per day at the inlet and outlet of the barriers.

Results and Discussion

It was found that the decolourization of the water solutions of dyes by means of bacteria related to the genus *Bacillus* was much more efficient in the cases when these bacteria were used in the form of washed cell suspensions (Table 2). The effect strongly depended on the pH of the reaction mixture (dye + bacteria) and much better results were achieved at acidic pH. This was due to the fact that the charge of the surface of the bacterial cells at pH higher than 4.5 was negative, like the charge of the dyes used in this study. However, the charge of the bacterial surface at acidic pH was changed to positive and this made the flocculation of dyes and the subsequent decolourization much more efficient.

Table 2. Treatment of water solutions of textile dyes by means of Bacillus spp. at pH ~3.3

Microorganisms	Reactive Black 5		Acid Orange 7		Disperse Yellow 5	
	I	II	I	II	I	II
	Colour removal, %					
Bacillus megatherium	21.4	74.2	53.2	83.7	0	60.2
Bacillus subtilis	21.7	79.0	44.8	81.8	0	66.2
Bacillus circulans	58.1	64.4	75.0	83.7	65.3	80.6

Note: I – Treatment by means of spent nutrient media;
II – Treatment by means of washed cell suspensions.

The amount of biomass in the reaction mixture was also very important for an efficient decolourization of the water solutions of dyes. The data shown in Table 2 were achieved by means of mixtures containing ~ 750 mg/l bacterial biomass and 100 mg/l dye, i.e. ~ 7.5 mg per mg dye. An increase of the biomass/dye ratio from 7.5 to 15 increased the colour removal of the dyes by means of washed cell suspensions to over 90 %. The individual *Bacillus spp.* differed considerably from each other with respect to their efficiency towards the different dyes. The best results were achieved by means of *B. circulans* which produced large amounts of exopolysaccharides. These exopolysaccharides formed the large mucilaginous capsules of these bacteria and partially dissolved in the nutrient medium. This explained the good results obtained by the spent nutrient medium formed after the cultivation of these bacteria.

The different dyes differed from each other with respect to their amenability to decolourization by means of bacterial treatment. The Disperse Yellow 5 was the most refractory to such treatment.

Apart from the flocculation, the sorption of dyes by bacterial biomass was also a mechanism involved in the decolourization of their water solutions. This was shown by the decolourization, although at a lower extent, achieved at pH close to the neutral point, as well as by experiments in which Mg^{2+} cations were added to the reaction mixture. These ions acted as desorbents towards ions, mainly bivalent, adsorbed on the bacterial surface and in this way increased the sorption capacity of this surface for the dyes. It must be noted also that different inorganic sorbents such as zeolite, bentonite and activated carbon were also active in the decolourization of dyes.

The decolourization of water dye solutions by means of an enriched mixed culture of flocculating bacteria was even more efficient that the decolourization achieved by means of the bacteria related to the genus *Bacillus* (Table 3). Very good results were obtained by means of spent nutrient media containing bacterial cells and secreted metabolites. This was connected with the large amount of biomass in these media (over 5 g/l). However, the best results were achieved by means of activated sludge used at high concentrations (> 3%) and a high biomass/dye ratio (Table 4).

Table 3. Treatment of water solutions of textile dyes by means of enriched mixed culture of flocculating bacteria

Dye	Way of treatment			
	By spent nutrient medium		By means of washed cell suspension	
	Initial pH of the system			
	3.2	6.5	3.7	5.3
	Colour removal, %			
Reactive Black 5	97.3	53.0	80.7	15.4
Acid Orange 7	97.2	86.8	79.6	52.5
Disperse Yellow 5	90.1	46.4	86.0	0

Table 4. Treatment of water solutions of dyes by means of activated sludge

Water solution of dye added to the activated sludge, ml	Reactive Black 5	Acid Orange 7	Disperse Yellow 5
30	98.0	98.0	90.5
50	95.2	96.1	90.3
80	99.1	96.3	91.23
100	97.0	97.4	92.5
150	99.1	96.1	87.3
300	99.5	95.0	84.4

The treatment of the dye solutions by means of permeable reactive barriers was very efficient (Table 5). It was found that the microbial cenoses which developed in the barriers contained mainly anaerobic and facultatively anaerobic bacteria. The pH during the treatment was stabilized around the neutral point and the redox potentials were slightly positive.

Table 5. Treatment of water solutions of dyes by means of permeable reactive barriers inoculated by activated sludge from wastewater treatment plant

Parameters	Reactive Black 5	Acid Orange 7	Disperse Yellow 5
pH	7.21-7.38	7.25-7.81	7.30-7.94
Eh, mV	(+8)-(+28)	(+35)-(+125)	(+5)-(+86)
Total dissolved solids, mg/l	91-125	95-442	1112-1175
Dissolved oxygen, mg/l	4.6-5.6	5.3-6.4	5.0-5.4
Dissolved organic carbon, mg/l	23-48	35-55	80-91
Heterotrophic aerobic bacteria, cells/ml	10^5-10^7	10^4-10^7	10^4-10^6
Heterotrophic anaerobic bacteria, cells/ml	10^4-10^6	10^4-10^6	10^4-10^6
Colour removal, %	80-88	86-91	78-84

The results obtained by means of the microbial treatment as a whole were similar to those obtained by means of coagulants, which are used in commercial-scale operations (Zetang 7101, 7102, 7103, 7197, 7125 which were supplied by CIBA Specialty Chemicals).

The decolourization of the dye solutions used in this study was stable and no coloration appeared again even after long periods of time (several weeks). This may be an indication for almost complete removal or degradation of the dyes. However, further studies are needed to establish the exact mechanisms involved in the solution decolourization. It is necessary to perform studies on the toxicity of the treated solutions to establish whether the colour removal is connected with a decrease of their toxicity.

Acknowledgements

This work was financially supported in the frame of the project N DAAD44 entitled "Mechanisms of bioflocculation and nature of the floccules and their industrial applications" in connection with the scientific cooperation between Germany and Bulgaria.

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