

Discoloration of azo dyes, Schäffer acid and R salt derivatives, by microorganisms of activated sludge

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Introduction

Dyes are very important water pollutant generally present in the effluents of the textile, leather, cosmetics, food processing, paper and dye manufacturing industries. An indication of the scale of the problem is given by the fact that over 100,000 commercially available dyes exist; more than 70,000 tons of these compounds are produced annually. During the dye manufacturing and dye application, up to 15% of the used dyestuff is released into the process water. As color is the first contaminant to be recognized in wastewater, the public perception of water quality is greatly influenced by the color. Dye effluents are also highly toxic to the aquatic organisms and they affect biological processes by reducing photosynthetic activity due to intensive coloration of water.

Most of actually used dyestuffs are the azo dyes [1]. Because of their complex chemical structure azo dyes are resistant to light, oxidizing conditions and microbial attack. Persistence of azo dyes, which is an advantage in the process of application and use, becomes a disadvantage in terms of biodegradation. Their resistance to biodegradation is due to a lack of analogues in nature (except for the 4,4'-dihydroxyazobenzene).

There are some treatment technologies for dye removal from wastewater such as coagulation, flocculation, membrane separations, ion-exchange, advanced oxidation processes and biodegradation, which are more or less efficient to eliminate dyes from aqueous solution [2, 3]. Most of them are very costly and commercially unattractive.

The most preferred, for economic and ecological reasons, it would be an application of biological methods to degrade azo dyes. One of the promising methods of increasing the effectiveness of biological decomposition of the dye molecule is its modification by introducing the rest of the sugar. This treatment does not affect the color and application properties of dyes, and it seems to be interesting because of the potentially easier biodegradability of the modified molecules. Colored azo compounds, containing in its structure the glucopyranose moiety and its derivatives, can also be characterized by lower toxicity [4] and mutagenicity [5]. They also show the high degree of exhaustion of the bath and bonding with the fiber, which reduces the amount of color wastewater. Dyes with the rest of D-gluconic acid have already been applied in the paper industry [6], as well as in the synthesis of fluorescent indicators and markers of cancer [7, 8].

Among the techniques of dye removal, adsorption gives very promising results as it can be used to remove different types of coloring compounds. If the adsorption procedure is designed correctly it will produce a high-quality effluent. Adsorption seems to be the most promising, alternative method of removing hard biodegradable compounds from wastewater.

Because of its great capacity to adsorb dyes, activated carbon is the most popular adsorbent but it also presents some disadvantages. It is quite expensive, non-selective, and ineffective against dispersed and vat dyes. It requires regeneration what results in a loss of the adsorbent [9].

Because the price is actually a very important parameter for comparing the absorbent materials, research is focused on various natural solid supports able to remove color pollutants from wastewater at low cost. Among the numerous cheap, non-conventional adsorbents proposed by several researchers, waste materials from industry and agriculture are worth to concern [10].

Biosorption of dyestuffs on microbial cells has received increased attention in recent years. Different types of organisms such as bacteria, fungi, yeast and algae are able to accumulate and degrade different pollutants [11–13].

Some organic substances (including azo dyes) which are resistant to biooxidation due to their chemical structure, can be adsorbed by activated sludge [10, 14]. The activated sludge technology is commonly used for a treatment of industrial and municipal wastewater. The sludge production in the European Union alone is more than 10 million tonnes per year. Due to low production costs, wide availability and high capacity of biosorption, activated sludge can be an excellent source of large amounts of a cheap adsorbent material [15].

The aim of our study was to investigate the effectiveness of living activated sludge of the removal of four azo dyes (two of them with glucopyranoside moiety) from aqueous solutions. The study included an effect of substituents in dye molecule and the impact of initial concentration of dye on the efficiency of this process.

Materials and methods

Four acid azo dyes used in this study (two of them with glucopyranoside moiety) were synthesized in Institute for Engineering of Polymer Materials and Dyes, Department of Dyes and Organic Products in Zgierz (presently Institute of Leather Industry in Łódź).

Activated sludge came from the full-scale sewage treatment plant in Łódź, purifying municipal wastewater of Łódź, Pabianice and Konstancinów Łódzki and used on the day of sampling.

The chemical structure of dyes is given in Figure 1.

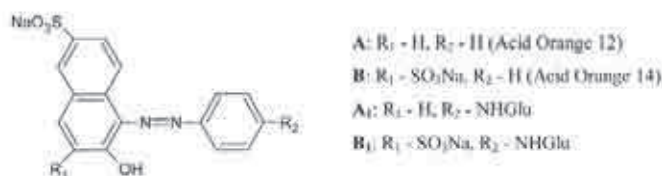


Fig. 1. Molecular structure of dyes and the type and position of substituents

The adsorption experiments were conducted in 500 ml beakers using 250 ml of activated sludge - dye solutions in aerated batch system. Initially, the concentration of dye of 100 $\mu\text{mol}/\text{dm}^3$ was used and the incubation time range from 15 min. to 24h. Controls, containing the same dyestuff but no biomass, were run in each experiment. The samples were taken at time intervals and centrifuged to remove solids from the liquid media. Decreasing dye concentrations were

measured on Hitachi U-2800 Spectrophotometer as the changes of the value of function $A = f(\lambda_{\max})$. The degree of dye discoloration was calculated as a decline in a value of absorbance of the dye solution relative to the control.

For the concentration-dependent adsorption studies the final concentrations of dyes in solution were 20, 50, 100 and 200 $\mu\text{mol}/\text{dm}^3$.

Results and discussion.

For the comparison of the spectral properties of examined dyes, their spectra in uv-vis in the range of 300-650 nm were made. The λ_{\max} and molar absorption coefficient were determined (Tab. 1.)

Table 1

Characteristics of the studied azo dyes

Dye	Molar mass	Purity, %	λ_{\max} , nm	ϵ , $\text{dm}^3/\text{cm}\cdot\text{mol}$
A	350	94.4	482	18 600
B	452	89.8	491	18 900
AI	538	100	496	19 300
BI	646	65.4	510	23 200

λ_{\max} – wavelength of maximal absorption, ϵ - molar absorption coefficient

Results of the discoloration of tested dyes after activated sludge treatment are shown in Figure 2. It was found that removing of color from solution strongly depends on a molecular structure of a dye. The highest degree of discoloration of dye solution (up to 55%), almost on the each stage of the experiment, was obtained for A₁ dye, Schäffer acid derivative with the sugar moiety in its structure. Additional sulfonic group in molecule decreased the efficiency of a dye removal. The worst absorption ability was observed for B dye (R salt) - with two sulphonic groups and no glukonic residue. It reached only 14.6% after 24 hrs contact with the activated sludge. An introduction to the molecule the D-gluconic acid moiety improved the effectiveness of discoloration of such modified dye particles by the activated sludge to 31.5%.

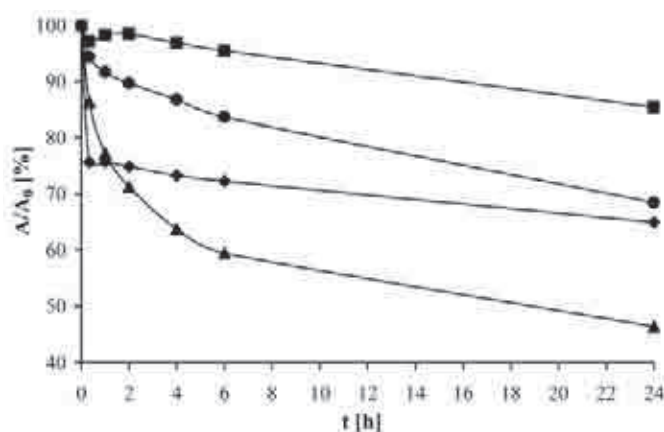


Fig. 2. Discoloration of azo dyes solutions treated with activated sludge during 24 h. (♦): A, (■): B, (▲): A₁, (●): B₁

The degree of removal of studied dyes by activated sludge is unsatisfactory. It is believed that the main mechanism for dispose of colored impurities in biological wastewater treatment by activated sludge is more adsorption on biomass rather than degradation [16, 17]. Tested substances belong to a group of acid

dyes and sulfonic groups present in molecule determine their good solubility in a water. Acid dyes are also called anionic dyes because of the negative electrical charge of the chromophore group. Activated sludge, on the other hand, has negative electrical charge in pH range from 3 to 10 [18]. Weak binding of the acid dye particles on the surface of the sludge is caused by electrostatic interaction between the negatively charged activated sludge, and negatively charged particles of dye. The greater the number of sulfonic acid substituents per molecule, the worse the efficiency of dye biosorption process, because the sulfonic groups increases the total negative charge of dye molecules, resulting in a stronger electrostatic repulsion with the negatively charged cell surface of activated sludge microorganisms. Because electrical forces are the main forces that drive adsorption, it is probably the main reason for the poor adsorption of acid dyes and worse result of adsorption ability of dye with two negatively charged groups.

The initial concentration also plays an important role in adsorption of dyes by activated sludge. Although this study have shown no increase in the degree of discoloration of solutions of dyes when changing the initial concentration (Tab. 2), but the total amount of adsorbed dye on the activated sludge increased. It means that dye adsorption capacity of activated sludge was increased with an increase in the initial dye concentration. The initial concentration of dye provides an important driving force to overcome all mass transfer resistance of the dye between the aqueous and the solid phase. Hence a higher initial concentration of dye will enhance the adsorption process [19].

Table 2

Degree of removal (%) of azo dyes from solutions and amount of dye adsorbed on the activated sludge after 24 h

Dye	Initial concentration, $\mu\text{mol}/\text{dm}^3$							
	20		50		100		200	
	%	q, mg/g	%	q, mg/g	%	q, mg/g	%	q, mg/g
A	36.4	0.6	35.7	1.5	35.8	3.1	41.6	7.3
B	7.8	0.2	10.1	0.6	9.5	1.2	9.5	2.4
AI	44.2	1.1	44.2	2.8	45.0	5.5	41.3	10.3
BI	27.2	0.7	21.5	1.4	22.8	3.1	21.8	5.9

q – amount of the adsorbed dye

In the case of dye B, when the lowest concentration used, the effect of initial increase in absorbance of the solution to about 106% of baseline was observed (Fig. 3). This is probably due to the breakup of dye aggregates caused by the activated sludge enzymes (Fig. 4). Anionic dyes are molecules containing hydrophilic (e.g., polar hydroxyl groups, amino groups) and hydrophobic parts (aromatic rings). In addition they may also contain groups that determine their solubility in water (sulfonic groups). Aggregation of sulfonated azo dyes in water occurs at relatively high concentrations and involves the creation of hydrogen bonds between hydrazo groups. In this way, molecules limit the interactions of hydrophobic groups with water and reduce the entropy of the system [20]. Hydrophobic effect is considered to be the main cause of the formation of dimers and larger aggregates [21]. For other dyes, no such effect was observed.

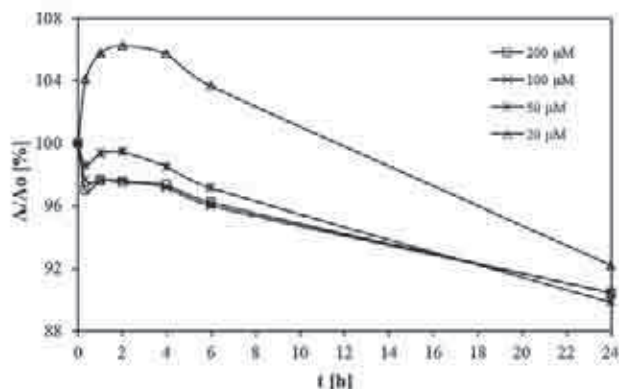


Fig. 3. Effect of initial concentration of dye B on the degree of discoloration of its solution treated with activated sludge for $t = 0 \div 24\text{h}$

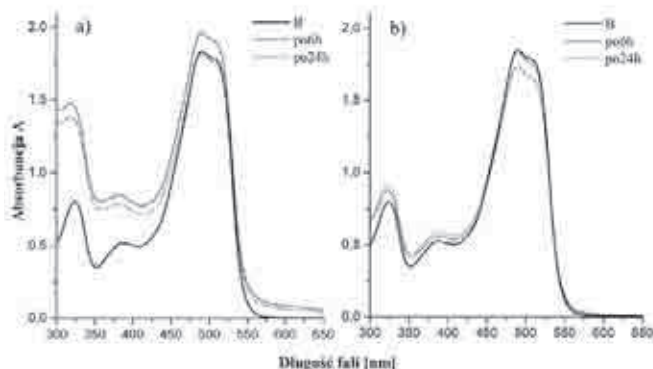


Fig. 4. Changes in the spectrum of dye B treated with activated sludge for $t = 0 \div 24\text{h}$. a) - $C_0 = 20 \mu\text{mol/dm}^3$, b) - $C_0 = 100 \mu\text{mol/dm}^3$

Summary

Type and number of substituents play an important role in the biosorption of investigated azo dyes. In the case of dyes with one sulfonic group (A and A₁) the obtained degree of solution discoloration was much better than for dyes with two sulfonic groups (B and B₁). The sugar moiety in the dye molecule also has a strong influence on the effect of dye discoloration. Higher degree of discoloration was achieved for dyes with the rest of the sugar (A₁ and B₁) than for the corresponding dyes without this substituent (A and B).

Initial concentration of dye has a significant impact on the values of adsorption capacity of activated sludge. For all investigated dyes, adsorption capacity of adsorbent increased while initial concentration of dye solution was increasing.

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