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THE PRELIMINARY STUDIES OF APPLICATION THE ALGAE *Mougeotia* sp. FOR THE REMOVAL OF SYNTHETIC DYES

BADANIA WSTĘPNE ZASTOSOWANIA GLONÓW *Mougeotia* sp. W USUWANIU BARWNIKÓW SYNTETYCZNYCH

Abstract: Biological methods of removal of synthetic dyes are cost effective and environmentally safe but still need improvement. The preliminary studies were focused on possibility of application of the freshwater algae *Mougeotia* sp. for the removal of synthetic dyes belonging to different chemical groups. Decolorization of water solutions of six dyes from four chemical groups were studied: azo – Evans blue (EB) and Congo red (CR); triphenylmethane (TPM) – brilliant green (BG) and crystal violet (CV); fluorone – bengal rose (BR) and anthraquinone – remazol brilliant blue R (RB). Dyes were used at three concentrations 0.005, 0.025 and 0.05 g/dm^3. The best results of dyes removal were reached in case of both triphenylmethane dyes (BG and CV) and fluorone BR for all used dyes concentrations (97–100 %). Increase of azo dyes concentration was connected with the decrease of the removal efficiency (CR – 68–100 %; EB – 37–87 %). In case of the antraquinone RB the inverse effect was observed and the best removal results were reached at higher dye concentrations. Preliminary results point out the possibility of use *Mougeotia* sp. in processes of synthetic dyes removal, but further studies are required.

Keywords: *Mougeotia* sp., decolorization, azo dyes, antraquinone dyes; triphenylmethane dyes, fluorone dyes

Introduction

Increasing requirements of consumers focused on the aesthetic quality of products are generating among others the increasing requirement of synthetic dyes. The ease and low costs of manufacturing as well as the wide range of offered colours contribute to the common use of large amount of this substances [1–3]. Synthetic dyes belong to the emerging pollutants (EPs). A significant number of them are introduced into the aquatic

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environment. The synthetic dyes, similarly as the rest of EPs, are not commonly monitored. They enter the aquatic environment and causing the adverse ecological and human health effects what is a serious worldwide concern. Their presence in aquatic environments affects the whole living ecosystems due to their frequent toxicity, carcinogenicity, resistance to biodegradation what is connected with their xenobiotic nature. Technologies focused on the decrease of their adverse environmental impact are divided into three categories – physical, chemical and biological [4–8].

Most of biological dyed wastewater treatment technologies use the living or dead biomass of bacteria and fungi. Among the bacteria species with the high decolorization potential the genera most often listed are: *Pseudomonas*, *Aeromonas*, *Bacillus*, *Citrobacter*, *Klebsiella*, *Burkholderia*, *Acinetobacter*, *Enterobacter* and others, and among the fungi these are the genera *Trametes*, *Pleurotus*, *Phanerochaete*, *Polyporus* [9, 10]. The efficiency of bacteria and fungi dyes removal processes depends on the type of used microorganisms, as well as the process conditions. The most important factors are temperature, pH value, oxygenation, available carbon and nitrogen sources, dye concentration and its chemical structure [9–14].

The application of algae and higher plants are rarely investigated. Studies on synthetic dyes decolorization are focused on the increasing of processes efficiency through their optimization as well as on the searching and estimation of possibility of using new organisms [7, 15–18].

It was found that some species of algae are able to remove various organic pollutants including synthetic dyes. Among the algae species from various groups are *Oscillatoria* sp., *Phormidium* sp., *Spirulina* sp., *Chlorella pyrenoidosa*, *Chlorella vulgaris*, *Spirogyra* sp., *Scenedesmus* sp., *Chara vulgaris* [15–19]. The abilities of removal of synthetic dyes by algae as well as bacteria or fungi species are different and depend on various factors. The properly conducted screening allows to find the species which possess the higher decolorization potential than others. The studies conducted on removal of synthetic dyes by various algae species showed that the effectiveness of process depends on kind of used dyes, their initial concentration, the pH value and the species of applied algae [15–19] El-Sheekh et al. [15] studied the decolorization abilities of six species of algae *Chlorella vulgaris*, *Lyngbya lagerlerimi*, *Nostoc lincki*, *Oscillatoria rubescens*, *Elkatothrix viridis* and *Volvox aureus*. The comparison of results of removal of azo (Congo red) and triphenylmethane (methyl red, orange II) dyes showed the higher efficiency of decolorization of triphenymethane than azo dyes (after 7 days of process the obtained removal results ranged between 4.02–81.97 % and 3.25–59.12 % respectively). Furthermore it was observed that the decolorization abilities of applied species differed significantly in dependence on the kind of dye. For instance the species *Oscillatoria rubescens* removed 81.3 % of TPM methyl red, 33.63 TPM orange II and 9.6 % azo Congo red, whereas *Chlorella vulgaris* removed 4.02 %, 43.68 % and 59.12 % respectively. In spite of the differences mentioned above the algae are perceived as promising group for application at environmental cleaning processes [15–19].

The present preliminary studies were focused on the assessment of the possibility of removal of some synthetic dyes belonging to various chemical groups by the common freshwater green algae *Mougeotia* sp.

Materials and methods

Dyes used in studies and solution preparation

Six different synthetic dyes belonging to four chemical groups were used in studies:
- azo – Evans blue (EB) and Congo red (CR);
- triphenylmethane (TPM) – brilliant green (BG) and crystal violet (CV);
- fluorone – bengal rose (BR);
- and anthraquinone – remazol brilliant blue R (RB).

All dyes were purchased in Sigma-Aldrich.

Dyes characteristics, including experimentally determined spectrophotometric properties (spectrophotometer UV-Vis Hitachi 1900) are presented in Table 1.

Table 1

Dyes used during decolorization studies – characteristics (based on [20] and the own data)

Azo dyes			
Evans blue			
Molecular formula: $C_{34}H_{24}N_6Na_4O_{14}S_4$	Molecular weight: 960.79 g/mol	Nr C.I.: 23860	Absorbance – $\lambda_{max.}$ [nm]: 606
Congo red			
Molecular formula: $C_{32}H_{22}N_6Na_2O_6S_2$	Molecular weight: 696.67 g/mol	Nr CAS.: 573-58-0	Absorbance – $\lambda_{max.}$ [nm]: 490
Triphenylmethane dyes			
Brilliant green			
Molecular formula: $C_{27}H_{34}N_2O_4S$	Molecular weight: 482.63 g/mol	Nr C.I.: 42040	Absorbance – $\lambda_{max.}$ [nm]: 624
Crystal violet			
Molecular formula: $C_{25}H_{30}N_3Cl$	Molecular weight: 407.98 g/mol	Nr C.I.: 42555	Absorbance – $\lambda_{max.}$ [nm]: 590
Fluorone dye			
Bengal rose			
Molecular formula: $C_{20}H_2Cl_4I_4Na_2O_5$	Molecular weight: 1017.64 g/mol	Nr C.I.: 45440	Absorbance – $\lambda_{max.}$ [nm]: 548
Anthraquinone dye			
Remazol brilliant blue R			
Molecular formula: $C_{22}H_{16}N_2Na_2O_{11}S_3$	Molecular weight: 626.54 g/mol	Nr C.I.: 61200	Absorbance – $\lambda_{max.}$ [nm]: 593

Each of dye was used as the water solution. Dyes water solutions were prepared in sterile distilled water and before usage were mechanically sterilized (cellulose syringe filters, pore size 0.2 μm). After sterilization the real concentration of the dyes in the solutions were estimated spectrophotometrically. Before usage the dyes solutions were stored at fridge in the dark-glass bottles.

A three initial concentrations of dyes in samples were used: 0.005, 0.025 and 0.05 g/dm^3.

The *Mougeotia* biomass preparation and decolorization experiment

The biomass of algae *Mougeotia* sp. was taken up from private garden pond in Gliwice (south Poland). Before usage the algae biomass was carefully and gently cleaned from every impurities (water plant residues, water animals e.g. snails etc.) and were threefold rinsed with sterile distilled water. In the next step the surface water was gently removed from biomass by usage the sterile filter paper. Then the samples of algae biomass each of 0.4 g were weighted. Such prepared biomass of *Mougeotia* sp. was used for decolourization study. For this purpose glass jars by volume 50 cm^3 were prepared. Jars were filled with 40 cm^3 of distilled water and then were sterilized by autoclaving (121 ^{o}C for 15 min.). Into cooled samples were introduced a properly prepared and weighted algae biomass. The experiment was carried out in the sterile room, equipped with the periodical lighting system (12 h day/12 h night – the lamp LEDDY SMART 2 by AQUAEL, Led 6 W, 470 lm). Samples were incubated at room temperature (19–21 ^{o}C).

Prepared cultures of *Mougeotia* sp. were left for one week in aim of biomass adaptation to the new conditions. After this time the algae biomass was floated on the surface of water, kept the fresh green colour and seemed to be in good condition. Into such prepared samples the solutions of dyes were introduced.

The decolorization experiment was performed in triplicate. Each studied dye was introduced into suitable jars containing the biomass of algae. The dyes solutions were introduced in proper volumes allowing to reach the initial dye concentrations: 0.005, 0.025 and 0.05 g/dm^3. Samples were cultivated in the sterile room, equipped with the periodical lighting system as was mentioned above (12 h day / 12 h night, room temperature). The control samples were the jars filled with the water solutions of dyes in given concentrations without algae biomass. Control samples were treated in the same way as the decolorization samples. During all the time of experiment the water losses were supplemented with the suitable volume of sterile distilled water. Experiment was conducted for 7–20 days (depended on the observed decolorization results).

The dyes removal efficiency was estimated in after process solutions by the spectrophotometric measurements. The residual of dyes in samples were calculated on the basis of standard curves. The percentage of dyes removal were calculated according to the formula (1):

$$R = (C - D/C) \cdot 100 \ [\%] \tag{1}$$

where: R – percentage removal of dye; C – the content of the dye in the control sample (without of algae biomass) [mg/dm^3]; D – the content of the dye in the decolorization sample (sample with algae biomass) [mg/dm^3].

At the end of the experiment besides the decolorization efficiency the wellness of algae biomass was estimated visually (filaments appearance).

Results and discussion

Synthetic dyes are classified as emerging pollutants. They cause a serious ecological threats and potential risks to human health even at minor concentrations. Their presence in surface waters is a serious worldwide problem [5, 6, 8]. In recent years the studies on the removal of synthetic dyes from wastewater are focused among others on the improving the efficiency of biological methods as well as on the searching of new organisms efficient in these processes. One of the group of organisms which is considered for these processes are algae [12, 15, 16, 18, 19, 21].

The presented preliminary studies were focused on the possibility of using of the freshwater green algae *Mougeotia* sp. in removal different synthetic dyes from water.

Mougeotia sp. is a common green freshwater algae belonging to the order *Zygnematales*. This algae species has a wide physiological plasticity and is able to maintain its population in good condition under a wide range of water temperatures, nutrient concentrations, solar radiation, pH levels [22]. Such properties suggests that *Mougeotia* sp. may be a promising candidate for using in processes of removal of synthetic dyes from water solutions and dyed wastewater. Obtained preliminary results showed the potential of *Muogeotia* sp. in removal of six structurally different synthetic dyes belonging to four different chemical groups (azo, triphenylmethane, fluorone, anthraquinone dyes) (Fig. 1–2).

The best results of dyes removal (97–100 %) were reached in case of both TPM dyes (brilliant green (BG) and crystal violet (CV)) and fluorone Bengal rose (BR) for all used dyes concentrations.. The initial dyes concentrations determined the removal efficiency

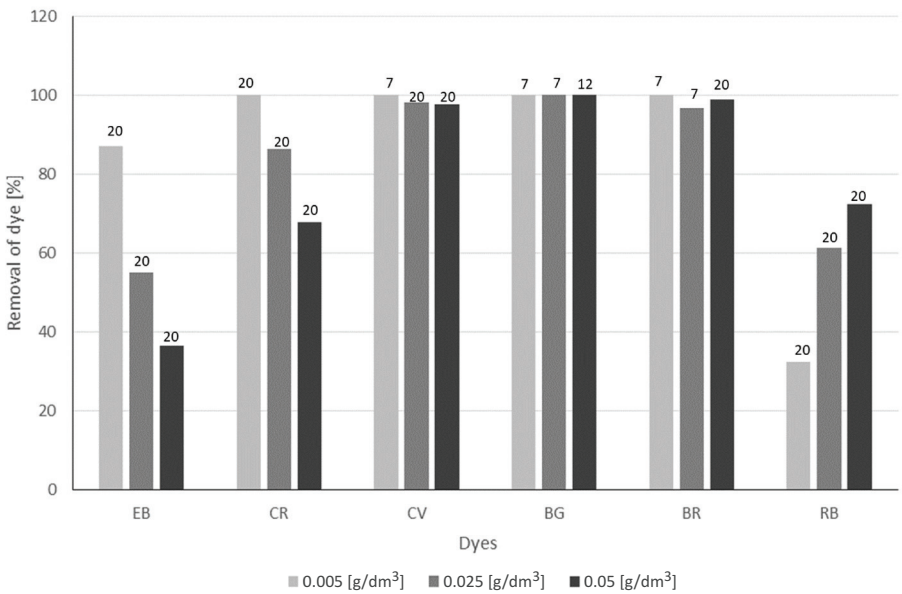

Fig. 1. Results of removal of different dyes used in various initial concentration by algae *Mougeotia* sp. (7, 12, 20 – the final day of experiment)

which was higher and needed less time in lower concentrations. All dyes listed above were completely removed after 7 days from samples with initial concentration 0.005 g/dm^3. From samples with concentration 0.025 g/dm^3 BG and BR were completely removed after 7 days and CV after 20 days. Increase of azo dyes concentration also was correlated with the decrease of their removal efficiency (in range of concentration from 0.005–0.05 g/dm^3 the removal was: CR 100–68 %; EB 87–37 % respectively). Mahajan and Kaushal [17] observed that rate of removal of azo dye Congo red by algae *Chara vulgaris* decreases with increasing dye concentration. The increase of CR concentration elongated the time needed for its removal. Such dependence was also observed by El-Sheekh et al. [15]. Decolorization abilities of different azo dyes applied in various concentrations by six species of algae (*Cyanobacteria – Lyngbya lagerlerimi*, *Nostoc linckia*, *Oscillatoria rubescens*; green algae – *Chlorella vulgaris*, *Elkatothrix viridis* and *Volvox aureus*) were studied. Dyes removal results were significantly better in lower concentration of dyes (5–10 ppm) and after 7 days of experiment ranged between 94.85–82.57 % and 93.3–82.15 % for *Cyanobacteria* and green algae respectively. In concentration 20 ppm the removal results were lower and ranged between 5.02–81.97 % and 3.25–59.12 % for *Cyanobacteria* and green algae, respectively [15].

In case of triphenylmethane BG and CV and azo EB and CR the deeply dyed biomass pointed on the significant participation of sorption in decolorization process (Table 2). Studies of use of algal-polymer biosorbent sheets in decolorization process showed a gradual decrease in methyl blue dye removal by increasing the dye concentration. This was a result of saturation of binding sites on the biosorbent material [7]. The biosorption efficiency is probably determined by composition of algal cell wall (increase in presence of reactive functional groups) [7].

In case of the antraquinone RB the inverse effect was observed (Fig. 1–2). The better removal results were obtained in samples with higher dye concentration (0.05 g/dm^3). Such results probably were due to static condition of cultivation (samples were not mixed) lack of sorption (biomass maintained natural green colour) and biotransformation as supposed main mechanism of decolorization (Table 2). Suspended and not mixed algae biomass probably was able to uptake dye more efficiently from solution with higher concentration than from sample with lower concentration where dye particles were more diffused. Pearce et al. [23] observed that low concentration of dye in solution makes difficult the recognition of dye by enzymes what cause the lower efficiency of decolorization process. Ergene et al. [16] for decolorization of remazol brilliant blue R used another green algae *Scenedesmus quadricauda*. It was shown that the uptake of RB increased with increasing initial dye concentration. The process stopped at higher dye concentrations when happened the binding sites saturation.

The chemical structure of dyes influenced on their removal efficiency (Fig. 1–2). This has been seen especially in the case of triphenylmethane dyes. In samples with dyes concentration 0.025 and 0.05 g/dm^3 the BG was completely removed after 7 days, while the elimination of CV needed 20 days. Probably it was due to the simpler chemical structure of BG which has two phenyl rings connected with two ethyl groups, while the CV is composed from three phenyl rings connected with three ethyl groups.

Similar dependence was observed in case of azo dyes, when structurally simpler CR was removed more efficiently than structurally more complexed EB (Fig. 2, Table 1).

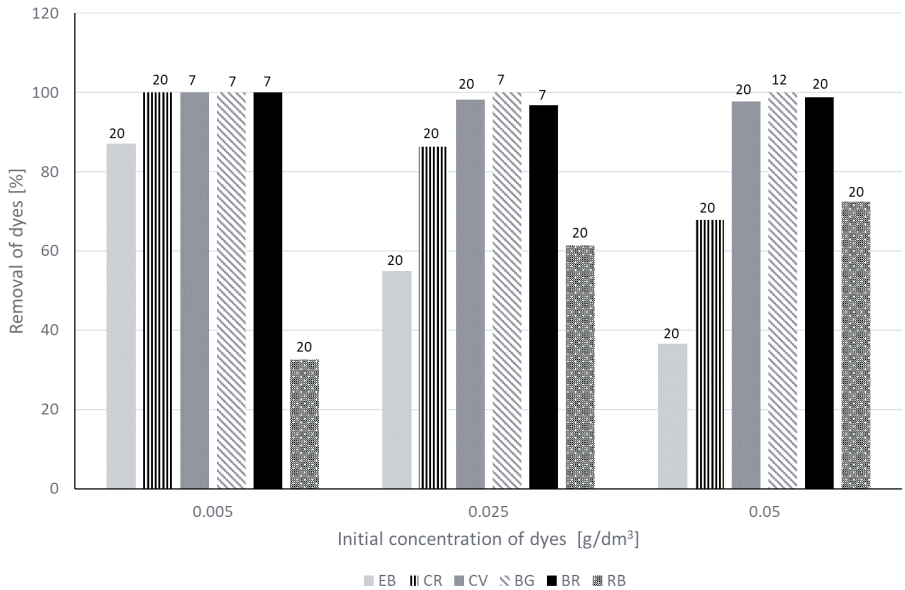

Fig. 2. The impact of dyes structure on its removal efficiency by algae *Mougeotia* sp. (7, 12, 20 – day of experiment)

Omar [24] studied decolorization of two azo dyes – monoazo Tatrazine and disazo Ponceau by different species of green algae (*Chlorella elipsoidea, Chlorella kessleri, Chlorella vulfgaris, Scenedesmus bijuga, Scenedesmus bijugatus, Scenedesmus obliguus*) and *Cyanobacteria* (*Anabaena laxa, Anabaena subcilindrica, Nostoc muscourm, Oscillatoria angusta, Nitzschia perminuta*). Obtained results pointed on the dependence of decolorization efficiency on the chemical structure of dyes. The disazo dye Ponceau was removed with lower efficiency than the monoazo Tatrazine which might be easier transported and/or adsorbed by algae cells because of the presence of charged sulphonic groups. These a strong electron withdrawing groups through resonance – caused and enhanced of colour removal.

The amount of colour removal varies with varying initial dye concentration [24]. Decolorization rate may depends on the structure and complexity of the dyes, particularly on the position of the aromatic rings resulting with the interactions with the azo bond as well as the number of azo bonds [12, 25, 26].

The preliminary studies did not allow us to determine the dominant mechanisms of removal process for sure. Although observation of samples and especially algae biomass suggests the participation of the sorption as well as the biotransformation process in dyes removal.

The cell wall matrix of green algae contains different functional groups such as amino, carboxyl, hydroxyl, phosphate and other charged groups connected with the

presence of the complex heteropolysaccharide and lipid components [16]. The biosorption efficiency is probably determined by presence of these functional groups in the cell wall [7, 16].

Table 2

Results of visual observation of growth and culture properties of samples with *Mougeotia* sp.at the end of decolorization experiment

Dye type	Initial dye concentration [g/dm^3]	The placement of Mougeotia biomass during/at the end of experiment (floating (F) or sedimented (S))	The colour of biomass of *Mougeotia* sp. at the end of experiment	Disintegration/changes of algae filaments
EB	0.005	S/F	blue	no
	0.025	S/S	blue	no
	0.05	S/S	blue	no
CR	0.005	S/S	red	no
	0.025	S/S	red	no
	0.05	S/S	red	no
CV	0.005	S/F	violet	thinner filaments
	0.025	S/S	violet	thinner filaments
	0.05	S/S	violet	thinner filaments
BG	0.005	S/F	deep green	disintegration
	0.025	S/F	deep green	disintegration
	0.05	S/F	deep green	disintegration
BR	0.005	S/F	natural	no
	0.025	S/F	natural	no
	0.05	S/F	natural	no
RB	0.005	S/F	natural	no
	0.025	S/F	natural	no
	0.05	S/S	natural	no

The visual observations of biomass of *Mougeotia* sp. during and after the decolorization process pointed on the changes in their appearance (Table 2). In all samples with dyes belonging to azo (EB, CR) and triphenylmethane (BG, CV) group the algae biomass was deeply dyed, and the colour maintained to the end of experiment. It suggest that sorption is the only/main mechanism of decolorization. The sedimented biomass in these samples (with the exception of BG) may confirm such mechanism of decolorization. High amount of dye adsorbed by biomass probably made it heavier, so caused the flotation impossible. Whereas the biomass flotation in samples with BG may be connected with their filaments disintegration, what made them lighter. At the end of experiment in samples with TPM dyes (for all used concentrations) was observed the adverse impact of these dyes on the biomass condition (the thinner algae filaments and even their disintegration (BG)). In samples with fluorone BR biomass was dyed at the beginning then returned to their natural green colour, what pointed on the participation of sorption as well as biotransformation in dye removal process. In case of antraquinone

RB the biomass maintained their natural colour at all time of experiment what suggest that the main mechanism of decolorization was biotransformation.

Preliminary results in most cases confirmed the possibility of use *Mougeotia* sp. in processes of synthetic dyes removal, but further studies are required.

Conclusion

The main reason of the presence of synthetic dyes in an aquatic ecosystems are ineffective wastewater treatment technologies. It causes a serious ecological problems and is on a high concern. This issue needs new solutions. Presented studies were focused on the assessment of the possibilities of using the freshwater algae *Mougeotia* sp. Obtained results showed that efficiency of dyes removal by *Mougeotia* sp. depends on many factors. The most important is type of used dye (chemical group and dye structure) and initial dye concentration in solution. The efficiency of removal ranged from 32.5 % to 100 %. The best removal results (total or almost total removal of dye) in the shortest time (after 7–12 days) were obtained in case of triphenylmethane BG (for all used concentrations), CV (in concentration 0.005 g/dm^3) and fluorone BR (for concentrations 0.005 and 0.025 g/dm^3). Dependence of removal efficiency on the dyes structure was observed. Removal of triphenylmethane CV, structurally more complicated than BG, needed more time. The same was observed in case of azo dyes when structurally simpler CR was removed more efficiently than EB. After 20 days of treatment all used dyes were removed in more than 50 % (with exception EB 0.05 g/dm^3 and RB 0.005 g/dm^3). Observations of physical condition as well as changes of colour of algae biomass (during and at the end of experiment) pointed on the participation of sorption in the decolorization process (dyed biomass) as well as biotransformation (returning or maintaining its natural colour). The nature of the process depended on the analyzed dye.

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