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Removal of Acid orange 7 by industrial lemon balm and yarrow solid wastes in dynamic conditions

M. Topuzova, I. Hambarliyska, A. Slavov*

Department of Organic and Inorganic Chemistry, Technological Faculty, University of Food Technologies, 26 Maritsa Blvd., Plovdiv 4000, Bulgaria

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Melissa officinalis L. (M) and *Achillea millefolium* L. (Y) residues of industrial steam distillation were used as cheap, abundant and eco-friendly adsorbents for removal of the azo dye Acid orange 7 (AO 7) from aqueous solutions in dynamic conditions. The biomasses had 75.26±0.98% and 80.39±1.37% of dietary fibers (DF) for M and Y, respectively, and the amounts of soluble DF were 4.59±0.57% and 5.04±0.43%, respectively. The polyuronic acid content for both plant materials was statistically non-distinguishable: 14.98±0.86% for M and 15.48±1.04% for Y. The dynamic adsorption of AO 7 was investigated at five different pH values: 1.21 (0.1 N H_2SO_4), 3.63 (deionized H_2O), 5.49 (50 mM citrate buffer), 8.30 (50 mM phosphate buffer), and 12.80 (0.05 N sodium hydroxide). Preliminary washing of M and Y biomasses with 0.1 N HCl increased their removal efficiency compared with water- and acetone-washed biomasses. Within the pH range from 1.21 to 5.49 the AO 7 removal was above 93% for M and 94% for Y biomass. The present work demonstrated that residual agricultural biomasses (M and Y) are able to remove the azo dye AO 7 in dynamic conditions (column adsorption) from aqueous solutions with an efficiency of 93.2±0.3% and 94.3±0.5%, respectively.

Keywords: Acid orange 7, azo dye, lemon balm, yarrow, column adsorption, dynamic adsorption.

INTRODUCTION

The production of essential oils in Bulgaria is well developed and main products are world-known and recognized. Aromatic and medicinal species (belonging to families of *Rosaceae, Lamiaceae, Asteraceae*, etc.) had been grown industrially for centuries. Although the main essential oil plants are rose (*Rosa damascena* Mill.) and lavender (*Lavandula angustifolia* Mill.) [1, 2], a great number of aromatic species are grown besides them, such as lemon balm, chamomile, yarrow, hyssop, thyme, etc. Due to the low content of essential oil in the plants their industrial processing generates huge amounts of residual biomass. Generally, the producers cut out the biomass nearby distilleries, where it slowly degrades [2].

The essential oils of lemon balm (*M. officinalis*) and yarrow (*A. millefolium*) have been well known for their therapeutic benefits. Lemon balm (M) possesses calming and anti-inflammatory effects, while yarrow (Y) is valued for its antimicrobial and wound-healing properties [3]. During the extraction of the essential oils, significant amounts of by-products are generated, which are rich in fibrous materials. These residues may have potential applications as adsorbents for pollutants, such as synthetic dyes. While previous research has investigated utilization of agricultural by-products like tea waste, sawdust, and peanut shells for dye adsorption, the potential of essential oil industry residues from lemon balm and yarrow remains largely unexplored [4].

Dye pollution is a pervasive environmental issue with significant impacts on both ecosystems and human health. Most synthetic dyes used in industries such as textiles, food, and leather have aromatic structures and contain chromophore functional groups (as azo group (-N=N-)), which enhance their stability and give them intense coloration. Owing to their complex molecular structures, these dyes persist in water bodies for prolonged periods, making biodegradation difficult [5]. Many synthetic dyes are harmful to aquatic organisms. They disrupt ecosystems by lowering oxygen levels, inhibiting the photosynthetic activity of aquatic plants, and reducing biodiversity [6]. Azo dyes like Acid orange 7 (AO 7) are particularly hazardous due to their persistent nature and toxic effects on aquatic species [7].

The chemical structure of AO7 features an aromatic ring attached to a charged sulfonic acid group and an azo linkage, making it highly water-soluble and resistant to natural degradation. Its chemical formula is $C_{16}H_{11}N_2NaO_4S$, and it is commonly present in industrial effluents, including its sodium salt form [7]. The azo group is particularly concerning due to its potential to break down under certain environmental conditions, forming aromatic amines, which are highly carcinogenic and mutagenic [5]. For these reasons

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* To whom all correspondence should be sent:
E-mail: *antons@uni-plovdiv.net*

waste waters containing azo dyes have to be purified before entering the water cycle.

Adsorption is among the most utilized methods for pollutant removal. Numerous materials were used as adsorbents [8, 9] and wastes or by-products (resulting from various industries) showed promising potential [10-14]. Static adsorption runs in batch mode and is easier to carry out compared to dynamic adsorption. Dynamic adsorption (column mode) could be operated continuously with the restriction of regularly changing the adsorbent. Lemon balm and yarrow biomass was employed for organic pollutant removal in static conditions [15]. The present work aimed at the utilization of these industrial residues as adsorbents for AO 7 removal in dynamic conditions (column adsorption).

MATERIALS AND METHODS

Materials

The lemon balm (*Melissa officinalis*) and yarrow (*Achillea millefolium*) residues were kindly provided by the distillery in Zelenikovo (Brezovo, Bulgaria, 2022; Galen-N Ltd.). Rose (R) (after steam-water distillation) and lavender (L) by-products (after steam distillation) were obtained from Galen-N (Zelenikovo distillery, region of Plovdiv, Bulgaria, 2022 harvest). AO 7 (4-(2-hydroxy-1-naphthylazo) benzenesulfonic acid sodium salt) was purchased from Merck (Germany). Activated carbon was obtained from Himtex Ltd. (Dimitrovgrad, Bulgaria). A chromatography glass column with support and PTFE stopcock (20×400 mm, NS 29/32; Isolab, Germany) was employed for dynamic adsorption.

Methods

The residues were crushed with a garden shredder and further milled with a grain milling machine. The biomasses were washed with deionized water, acetone and 0.1 N HCl as described [16]. The AO 7 solutions (1 mg/mL) at different pH were prepared according to Wu *et al.* [17].

Dynamic adsorption was carried out in a 400 × 20 mm column, NS 29/32 (Isolab, Germany), equipped with a crucible filter at the lower end and a Teflon stopcock. 5 g of adsorbent were placed in the column and around 2 g of cotton were used at the upper end to prevent layer disruption during the addition of the liquid phase. 100 mL of the azo dye solution were gently poured, and after 30 min, 4 mL-fractions were collected in 5 mL Eppendorf tubes. Each fraction was filtered using filter paper and subsequently syringe filter (CA 0.45 μm, Isolab, Germany). The adsorption was assessed using an LLG-uniSPEC 2 UV-Vis spectrophotometer at 500 nm (LLG Labware, Germany). The concentration of AO 7 was calculated using a standard curve prepared with solutions of the azo dye of known concentrations.

The proteins' amount was assessed by the method of Kjeldahl (MultiKjel K-365 – Büchi, Switzerland). The PUC and DE of the biomasses were determined as described in [16]. The moisture content was determined with KERN DAB 100-3 analytical balance (KERN&SOHN GmbH, Germany). The total, insoluble and soluble dietary fibers (TDF, IDF and SDF, respectively) were determined using K-TDFR-100A (Megazyme, Ireland), according to AOAC method 991.43 "Total, soluble and insoluble dietary fibers in foods" (First action 1991) and AACC method 32-07.01 "Determination of soluble, insoluble and total dietary fibers in foods and food products" (Final approval 10-16-91). The ash was determined by igniting 1 g sample placed in crucible in a muffle furnace at 605 °C until constant weight.

Statistical analysis

The experimental data (three replicates) are presented as mean value ± standard deviation. For analysis one-way ANOVA test (Tukey's post hoc test; $p < 0.05$) was used with Microsoft Excel 2013 (additional XL Toolbox NG module installed).

RESULTS AND DISCUSSION

Chemical and physicochemical characteristics of M and Y

The amounts of total dietary fibers (TDF) in M and Y residues were 75.26±0.98 % and 80.39±1.37 %, respectively, while the amounts of soluble dietary fibers (SDF) were relatively low: 4.59±0.57 % (M) and 5.04±0.43 % (Y). The insoluble dietary fibers (IDF) fraction predominates (68.50±0.88 % for M and 74.87±1.19 % for Y) and it includes mostly cellulose, hemicelluloses and lignin. The protein content in both biomasses was statistically not significant: 6.69±0.24 % (M) and 6.33±0.38 % (Y). The polyuronic acid content (PUC), commonly referred to acidic polyuronic biopolymers in the plant matrix, was around 15 %, and the degree of esterification (DE) – above 85 % (Table 1). Figure 1 presents information from the mesh size analyses of the biomasses. The predominating fractions were in the 0-150 μm and 450-670 μm size range for M and Y residue, respectively.

Table 1. Chemical characteristics of M and Y biomasses

	TDF, %	IDF, %	SDF, %	DE, %	PUC, %	Ash, %	Protein, %
M	75.26±0.98^{b}	68.50±0.88^{b}	4.59±0.57^{a}	85.60±3.56^{a}	14.98±0.86^{a}	8.66±0.38^{a}	6.69±0.24^{a}
Y	80.39±1.37^{a}	74.87±1.19^{a}	5.04±0.43^{a}	87.20±2.47^{a}	15.48±1.04^{a}	5.96±0.06^{b}	6.33±0.38^{a}

Results are presented as the mean of three measurements; ^{a,b} Different letters in columns indicate statistically different values (One way ANOVA with post-hoc Tuckey's HSD test, $p < 0.05$).

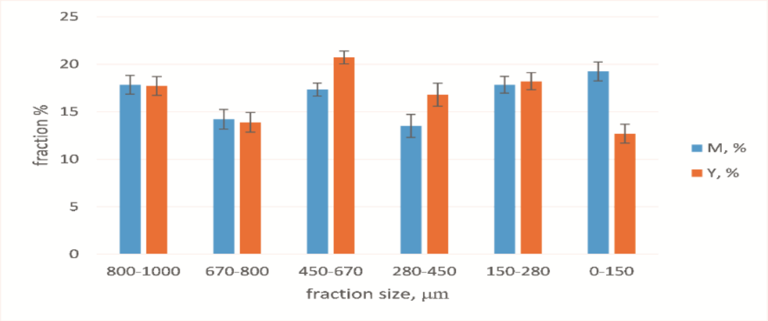

Figure 1. Particle size analysis of M and Y

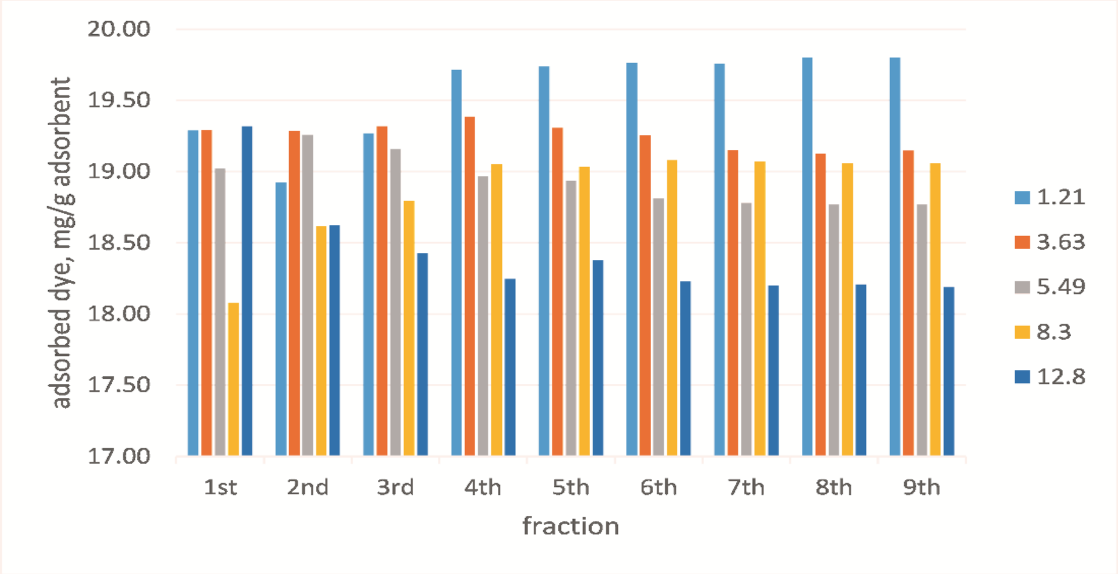

Figure 2. Adsorption of AO 7 at five different pH values on M residue washed with water

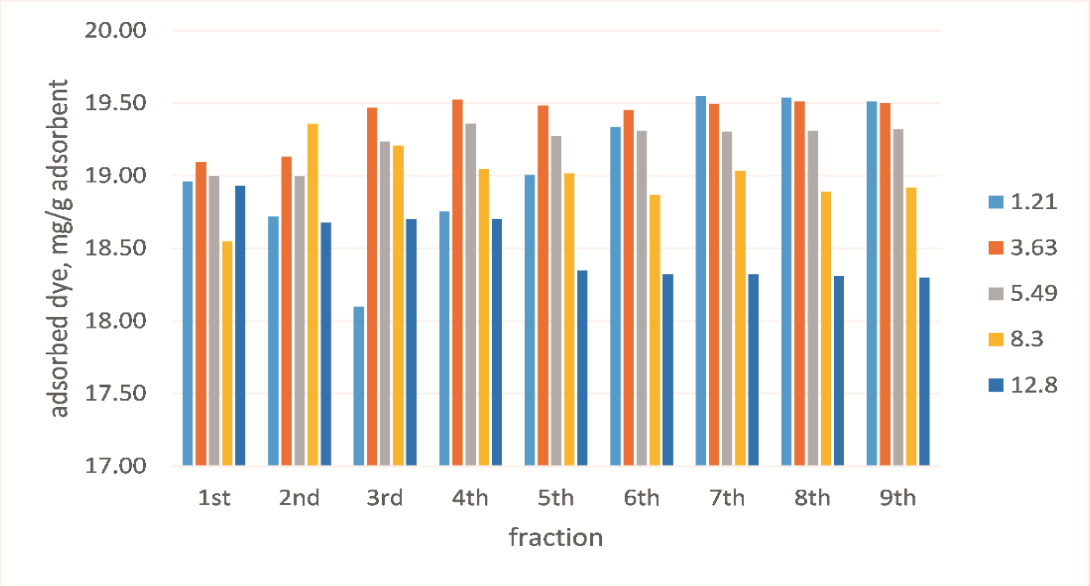

Figure 3. Adsorption of AO 7 at five different pH values on M residue washed with acetone

Contact time and pH influence on AO 7 dynamic adsorption

The residues were pretreated with deionized H_2O, acetone and 0.1 N HCl. These pretreatments were made to extract low-molecular substances from the biomasses. Washing with 0.1 N HCl led to changing the functional groups' charge: carboxylic groups will be neutralized while amino groups will get positive charge. It is expected that this positive charge will contribute significantly to the adsorption having in mind that AO 7 is bearing negative charge.

Adsorption of the dye was performed at five different pHs (from strongly acidic – pH 1.21 to basic – pH 12.80 conditions). The experimental results for utilization of M residues as adsorbent are presented in Figures 2-4. The process relies on physical and chemical interactions between biomasses and dye. The predominating functional groups in the adsorbent and the compounds adsorbed play vital role and determined the effectiveness of the process. These interactions are stronger and adsorption is fast in the beginning (first collected fractions) because the unoccupied groups on the surface of the adsorbent predominates [12]. The data presented suggested that lower pH (5 or less) favors adsorption effectiveness (Figures 3 and 4). The highest percentage of removed AO 7 was observed at lower pH (1.21) with acid-pretreated M residue: above 93% of removed dye. The water-washed M biomass showed better removal at pH 1.21. At higher pH (12.80) the effectiveness dropped and this tendency was observed for all pretreated M residues.

In the next experiments the Y residue was explored as adsorbent in dynamic conditions for AO 7 removal (Figures 5 to 7). Similar conclusions as for lemon balm residues could be drawn. The water-washed Y biomass exhibited the highest adsorption efficiency at lower pH till the 5th fraction collected while hydrochloric acid-washed Y showed slightly better performance at pH 3.63. In all cases, above pH 8.3 the effectiveness was low. Above this pH the percentage of adsorbed AO 7 was the lowest. Comparable findings and assumptions were made by Marovska *et al.* [12], Wu *et al.* [17] and Hambarliyska *et al.* [15] investigating the removal of β-naphthol orange (static conditions) employing rose and lavender (residues from industry for essential oil production), brewery spent yeasts, and lemon balm and yarrow wastes. Collection of fractions beyond 5th and 6th for both adsorbents showed that the processes of adsorption/desorption are entering equilibrium and it is difficult to achieve better removal of the AO 7.

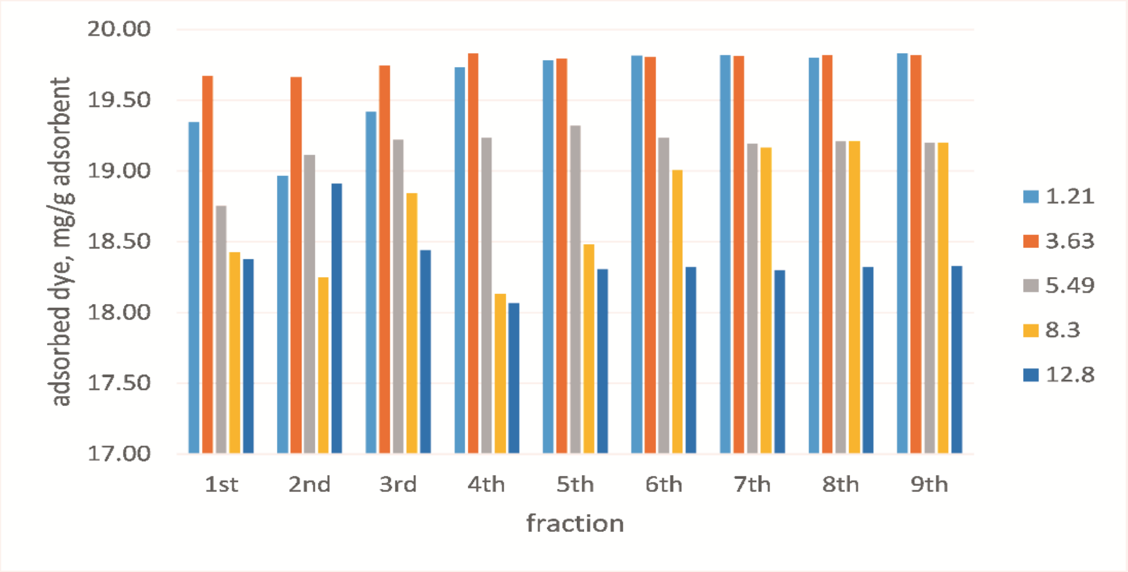

Figure 4. Adsorption of AO 7 at five different pH values on M residue washed with 0.1 N HCl

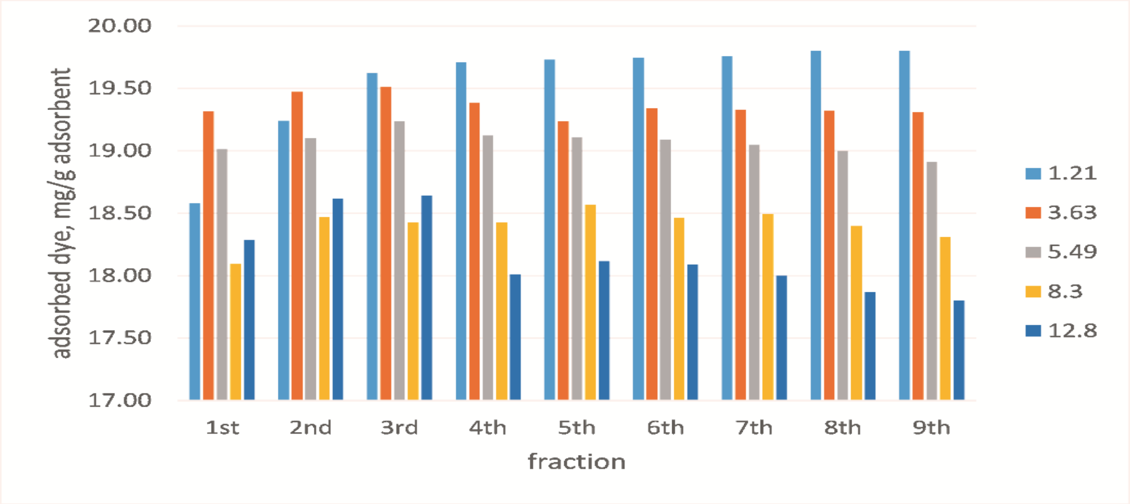

Figure 5. Adsorption of AO 7 at five different pH values on Y residue washed with water

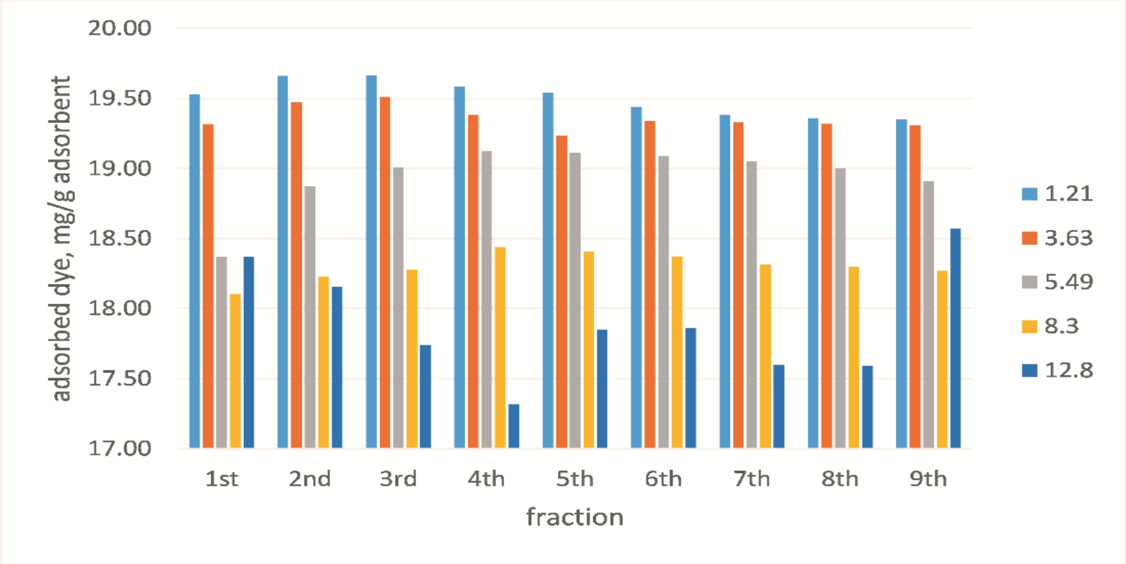

Figure 6. Adsorption of AO 7 at five different pH values on Y residue washed with acetone

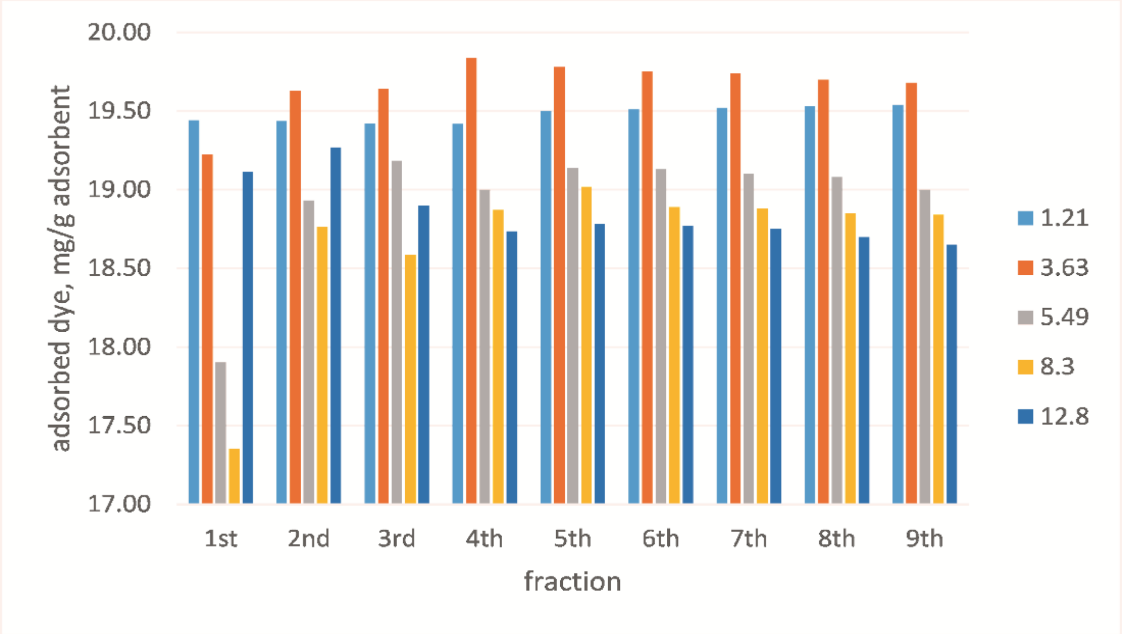

Figure 7. Adsorption of AO 7 at five different pH values on Y residue washed with 0.1 N HCl

Thereafter, a comparison of the effectiveness of AO 7 removal between the M and Y biomasses (water-washed) and rose and lavender post-distillation residues (water-washed) was performed. As a positive control was used activated carbon – a versatile and well known adsorbent. The results are presented in Figure 8.

The activated carbon, as expected, showed the best effectiveness in AO 7 decontamination. The adsorption rate and effectiveness of M and Y for AO 7 removal (from aqueous solution) was comparable with that of rose residues (at acidic pH) and slightly lower than that of lavender residues. This tendency was kept for till pH 5.49 and at higher pH M and Y residues showed better effectiveness than R and L residues. The observed results could be tentatively explained with the predominating physical character of adsorption of the dye onto the biomasses and the time and pH necessary in the begining for increasing the effectiveness. Similar observations were reported by Hambarliyska *et al.* [15] investigating the removal of β-naphthol orange with lemon balm and yarrow residues in static conditions and by Marovska *et al.* [12] investigating the adsorption of β-naphthol orange using rose and lavender biomasses in static conditions.

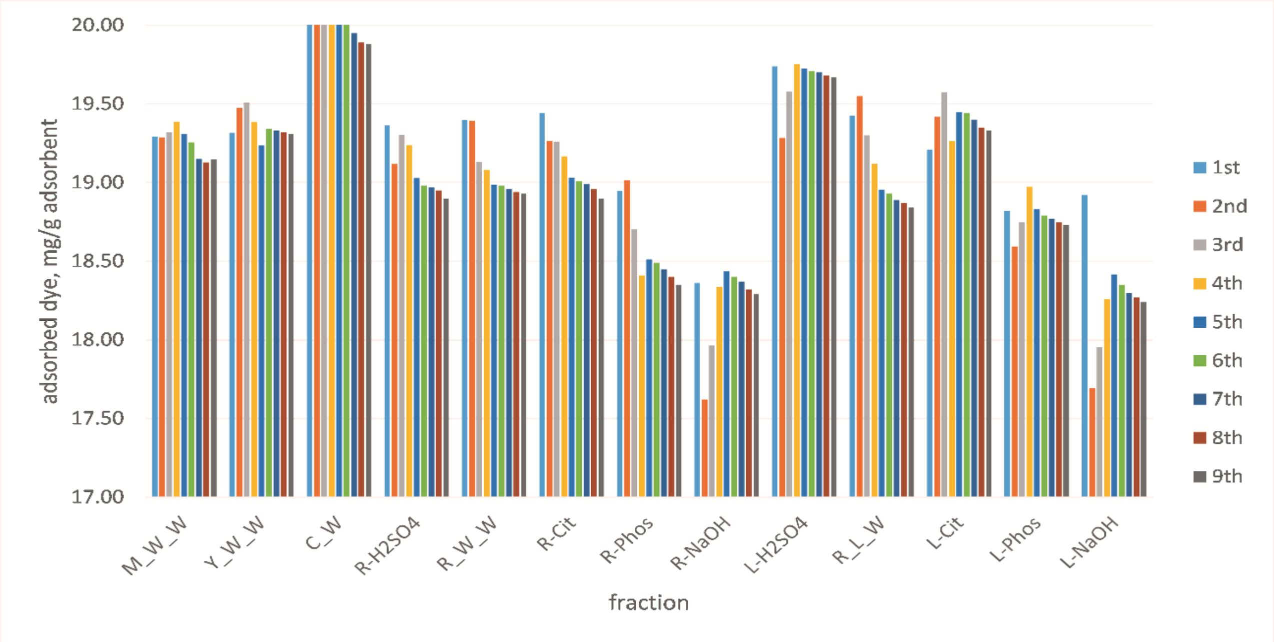

Figure 8. Comparison of AO 7 removal by M and Y residues (water-washed – M_W_W and Y_W_W) with C_W – activated carbon, rose (R) and lavender (L) residues (solutions at five different pH: 1. pH 1.21 – 0.1 M H_2SO_4; 2. pH 3.63 – aqueous dye solution; 3. pH 5.49 – 50 mM citrate buffer; 4. pH 8.30 – 50 mM phosphate buffer; 5. pH 12.80 – 0.1 N NaOH)

CONCLUSIONS

The present study investigated the removal of AO 7 (an azo dye) in dynamic conditions with two industrially generated biomasses – *M. officinalis* and *A. millefolium*. Residues pretreated with 0.1 N HCl demonstrated the highest adsorption capacity for AO 7 removal but the process was slower at the beginning of the adsorption. An adsorption/ desorption equilibrium was observed after collection of the 5th and 6th fraction. The pH is among the most important factors affecting the adsorption effectiveness and for M and Y residues higher efficiency was observed below pH 5.5. The optimal conditions for AO 7 removal could be summarized as follows: pH below 5, initial pretreatment of the plant materials with dilute acids, and contact time at least 30 min. Comparison of the M and Y residues (water-washed) with activated carbon and biomasses of rose and lavender (also generated by the essential oil industry) suggested that lemon balm and yarrow biomasses have significant potential to be used as biodegradable and renewable adsorbents for azo dyes from their aqueous solutions.

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