Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2015.1070290

57 (2016) 15007–15019
July

Indigo dyeing wastewater treatment by eco-friendly constructed wetlands using different bedding media

Gamze Dogdu, Arda Yalcuk*

Faculty of Engineering and Architecture, Department of Environmental Engineering, Abant Izzet Baysal University, Gölköy Campus, Bolu, Turkey, Tel. +90 374 2541000 4906; Fax: +90 374 2534558; emails: dogdu.gamze@gmail.com (G. Dogdu), ayalcuk@gmail.com (A. Yalcuk)

Received 11 December 2014; Accepted 30 June 2015

ABSTRACT

Most natural and synthetic textile dyes, especially vat dyes, are resistant to degradation and decolorization by conventional treatment methods. In this study, the purification of synthetic textile wastewater containing commercial indigo dye by a green technological treatment system was investigated. A vertical-flow constructed wetland model comprising three different bedding materials, sand, gravel, and zeolite, was used to treat synthetic indigo dyeing wastewater. Treatment efficiency was evaluated by measuring color and pollution parameters such as chemical oxygen demand (COD), pH, oxidation–reduction potential, and electrical conductivity. According to the results, the constructed wetland system reduced color by up to 97% and lowered the COD by up to 62%. This study demonstrated that the constructed wetland system is a promising technique for purification of indigo dyeing textile effluents of COD and color as compared to conventional methods.

Keywords: Vertical-flow constructed wetlands; Vat dye; Indigo dyeing wastewater; Decolorization; COD removal; Zeolite

1. Introduction

The textile industry produces wastewaters of different compositions during manufacturing processes (dyeing, bleaching, printing, and finishing) containing a mixture of chemicals, auxiliaries, and various dyestuffs of different classes and chemical constitutions with organic parameters such as chemical oxygen demand (COD) and inorganic parameters such as metals, chloride, and nitrogen [1]. Several million tons of synthetic dyes including azo dyes, cationic and anionic dyes, vat dyes, dispersive, reactive, and indigoid dyes are manufactured every year across the world [2]. Textile dyes are resistant to fading upon exposure to heat, water, light, and oxidizing agents, making them stable and resist to biodegradation [3]. Colored wastewater is resulted by incomplete dye degradation. The presence of unnatural color is esthetically undesirable and associated with environmental pollution [4]. Color is a highly important contaminant in wastewater; a very small amount of dye in water (10–20 mg/l) is extremely visible and affects transparency and gas solubility of wastewater [5]. The presence of color, nutrients, and organic materials in wastewater is a problem and should be treated before discharging to the environment [6]. It is known that most dyes cannot be effectively treated prior to their discharge into environment [2]. Therefore, several

*Corresponding author.

physical, chemical and biological wastewater treatment methods are applied to treat textile effluent in order to meet regulatory discharge limits. However, common physicochemical and biological wastewater treatment methods cannot supply an adequate degree of degradation or color removal due to the chemical stability and resistance to microbial attack of the dyes [7–10]. Physical treatment methods such as coagulation, flocculation, and common sedimentation can be used to remove dispersed dyes and sulfur from wastewaters, although they lead to the production of sludge and are less effective for color removal [11]. Membrane filtration methods, ultra, nanofiltration and reverse osmosis are also used to remove color and organic matter. However, these methods are very expensive and suffer from rapid membrane fouling. Other treatment methods include advanced oxidation processes and usage of fungi and algae [12]. However, when all treatment methods are examined in detail, it is clear that they are not feasible for treating dye-rich wastewater because of intensive technology, constant power demand, complexity of components, unproven long-term effectiveness and high investment and maintenance costs [13].

Due to the drawbacks of the conventional treatment methods, in recent years, phytoremediation has become a promising technology for treatment of textile dyes and especially for industrial wastewaters using plant and microbial communities from the rhizosphere to eliminate various organic and inorganic chemical contaminants [1,14–18]. Constructed wetland systems are based on natural components (vegetation and microbes). These systems treat organic matter, nutrients, and suspended solids in domestic wastewater, leachate, agricultural, and industrial wastewater. Constructed wetland systems have many unique advantages such as economic aspects, easy usage, high removal efficiency of organic and inorganic matters, and environmental friendliness [19–22].

Indigo ($C_{16}H_{10}N_2O_2$), one of the oldest known blue dyes, is still employed extensively today for dyeing cotton yarn in the manufacture of denim and blue jeans. The current annual consumption of indigo and other vat dyes reaches about 33 million kg [23,24]. About 15% of the indigo used is lost during the dyeing process [25]. Indigo, classified a vat dye although its properties are not typical to the vat dyes as a whole, is abundantly used for denim manufacturing and in denim-dyeing plants [23,26]. Vat dyes have superior fastness properties to light, washing, and chlorine bleaching than indigo; however, it is this unique color fading that has kept it so popular [23]. Wastewater containing indigo is characterized by a dark blue color due to the presence of the dye not fixed to the fibers during the dyeing process [27]. Indigo is a so-called vat dye, which means that it needs to be reduced to its water soluble *leuco*-form before dying. The reduced form is absorbed into the fibers and when oxidized back to its blue form, it stays within the fiber [23]. Also, indigo is practically insoluble in water (2 ppm) and has no affinity for cellulose fibers in such a state. Thus, it has to be reduced using a powerful reducing agent such as sodium dithionite ($Na_2S_2O_4$) before dying in order to be converted into a water soluble form [28]. This process leads to the production of a high amount of wastewater, which must be treated with appropriate methods before discharge into the environment. Indigo has a low mammalian toxicity, and there is no indication of sensitization in humans after repeated skin applications. However, reduction with sodium dithionite is considered environmentally harmful and unfavorable due to wastewater-contaminating degradation products [23]. Indigo dye was chosen in our study for treatment by an ecological method in order to prevent environmental and health concerns thatare discussed above.

There are some studies on degradation of indigo dye solutions using an adsorption process with activated carbon [29], calcium hydroxide [2], ultrafiltration and nanofiltration [30], microfiltration [31], and electrochemical processes [33–36]. Also, there are some other recent reports on degradation of indigo dye wastewater using biological methods [37–40], aerobic treatment methods [41], anaerobic treatment processes [42], and physicochemical treatment methods [27,43]. However, these treatment techniques have many drawbacks that will be discussed in the following section of the manuscript. Hence, in this study, a cost-effective and eco-friendly constructed wetland system, which has not yet been studied at the field-application level, was used to treat indigo dyeing wastewater on the basis of decolorization and organic pollution reduction (COD).

2. Materials and methods

2.1. Synthetic textile wastewater

The dye used with the wastewater was commercial synthetic blue indigo dissolved in water (40%) containing some impurities in solution which was supplied from Realkom Denim Textile Factory in Düzce, Turkey to simulate realistic operating conditions. Fig. 1 shows the chemical structure of the original indigo dye. 1,000 ppm synthetic stock wastewater solution was prepared and diluted as 1:4 ratios to 4.5 L filled reactor volume. To examine the removal of commercial indigo, synthetic wastewater was only prepared with dye and diluted with tap water.

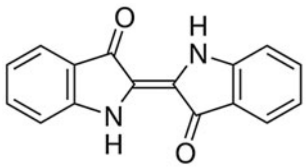

Fig. 1. Chemical structure of indigo.

2.2. Experimental setup and procedure

Three cylindrical PVC reactors with internal diameters of 20 cm and heights of 25 cm were used in the experimental study. *Canna indica* (R2) was planted (64 rhizomes/m^2) in one of the reactors and *Typha angustifolia* (R3) (64 rhizomes/m^2) in one of the others. The third reactor was left barren as control (R1). The treated outlet water was collected through a global valve that was placed 8 cm above the base level. Filters placed at the inner siding of the valve were used to prevent blockage by the filling material as well as the loss of the filling materials. The reactors' hydraulic characteristics are summarized in Table 1.

The synthetic textile vat dye containing wastewater was fed to the reactor systems permanently (for a duration of 5-min feeding/hour) by a peristaltic pump (Ismatec VC 280), which might have enabled air trapping within the porous filling material in between the wastewater feeding periods. Feeding was arranged as 1.2 L/d (0.5 L/h) that passed through the system providing hydraulic retention times of 3.75 d for each system (Fig. 2).

Sand, zeolite, and gravel were used as the filling materials in the reactors. The type of loading materials and media depths in the reactors is detailed in Table 2. Zeolite (clinoptilolite) was used in addition to the commonly used sand and gravel media in order to improve the removal mechanisms (ion exchange and adsorption) in the vertical constructed wetland systems.

According to previous reports, *Typha* and *Canna* plants can grow rapidly in small wetland conditions and have high domestic and industrial wastewater treatment efficiencies. *Typha* colonizes a large surface area and produces very high biomass yield [44]. *C. indica* is considered a phytoremediation plant, with a flourishing root system with higher root growth, higher root number, larger root biomass, and significantly larger root surface area than other plant species [45]. Therefore, in this study, *T. angustifolia* and *C. indica* plants were selected as the wetland plants.

Table 1
Hydraulic characteristics of the reactor systems

Reactor type	Filling volume *V* (L)	OLR (kg COD/m d)	HRT (d)	*Q* (L/d)
Control (R1)	4.5	0.067	3.75	1.2
C. *indica* (R2)	4.5	0.067	3.75	1.2
T. angustifolia (R3)	4.5	0.067	3.75	1.2

2.3. Analytical methods

The efficiencies of the systems were evaluated by measuring color and organic pollution. Plant lengths were measured periodically to ensure plant growth when they were treating organic–inorganic pollutants. Influent and effluent samples were analyzed for COD twice a week according to standard methods [46] and using Merck Spektroquant test kits together with a Pharo 100 spectrophotometer. pH, oxidation–reduction potential (ORP) and electrical conductivity (EC) were measured with Thermo Scientific Orion 5 Star series. The absorbance of the synthetic vat dye containing wastewater before and after treatment was measured at the wavelength of maximum absorption for commercial indigo dye (613 nm). Prior to absorbance measurement, the effluents were centrifuged for 10 min at 3,000 rpm to remove turbidity. The percentage of color reduction (CR) was calculated according to the following equation:

$$\text{CR }(\%) = \frac{A_0 - A}{A_0} \times 100. \quad (1)$$

where *A*0: initial absorbance, *A*: absorbance after treatment.

The biofilm layer was observed on the supporting media by scanning electron microscopy (SEM) before and after the experiments using a JSM-6390LV, JEOL scanning electron microscope. Functional group differences of commercial indigo dye were observed after the experiment using Shimadzu IRPrestige-21 FTIR Spectrophotometer at 400–4,000 cm^{-1}.

In this study, treatment performance of wastewater containing vat dye indigo by laboratory scale, economically designed vertical-flow intermittent feeding constructed wetlands planted with *C. indica* and *T. angustifolia* was investigated. Input and output samples were taken twice weekly over a 91-d experimental period following a week of planning and allowing acclimation of the plants to the environment. Prepared synthetic wastewater was diluted with tap water in the system due to reliability, low cost, and toxicity concerns to the plants.

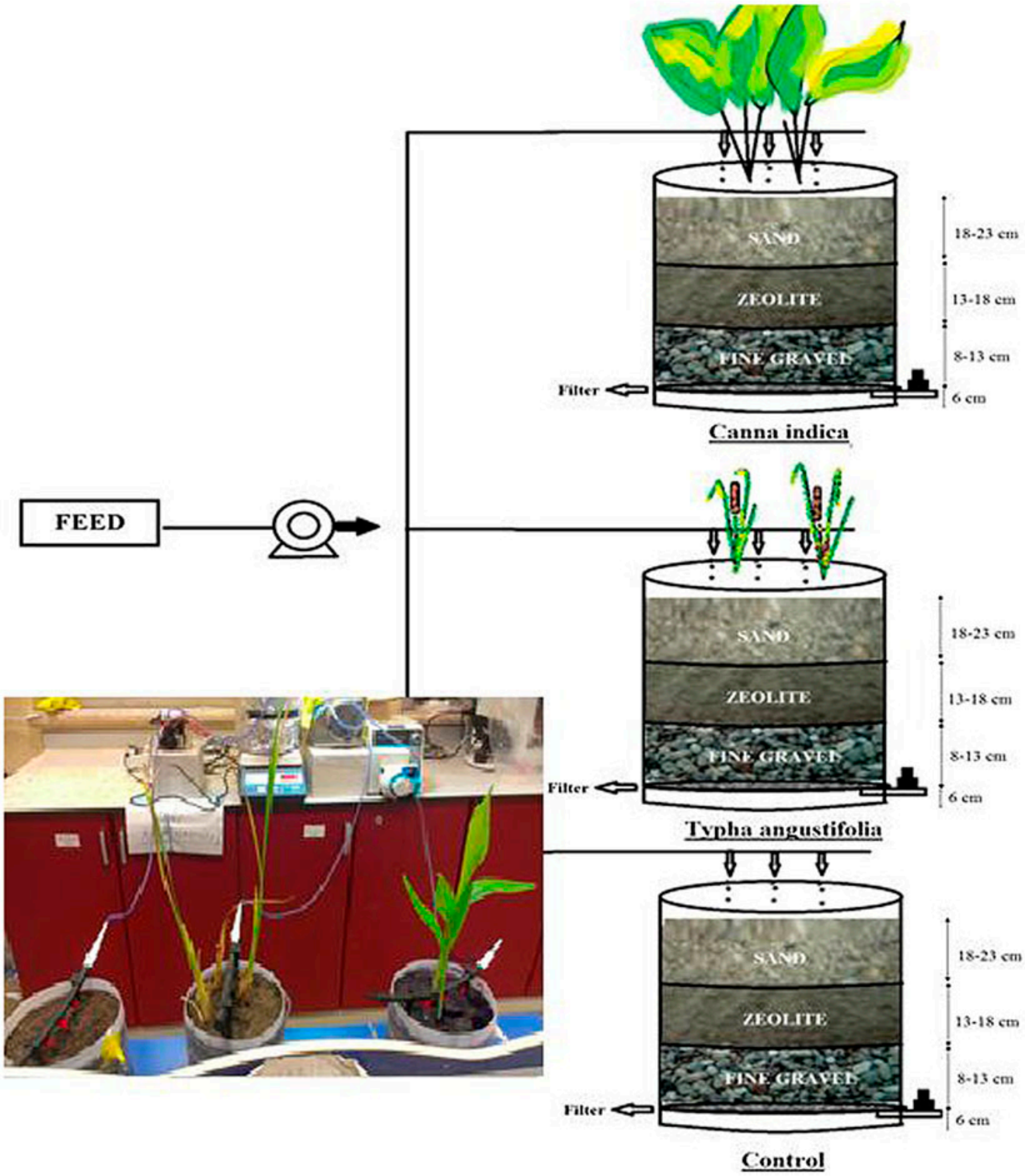

Fig. 2. Reactors were used in experimental studies.

Table 2
The type of the loading materials and media depths in the reactors

Filling materials	Material depth (cm)
Filter	0–8 cm
Fine gravel (7–15 mm)	8–13 cm
Zeolite (0.8–2 mm)	13–18 cm
Sand (0–7 mm)	18–23 m

2.4. Statistical analysis

In order to evaluate the wastewater treatment performance of R1, R2, and R3 reactors, discriminant function analysis was performed. One-way ANOVA (at a significance level of 0.05) was applied to the removal efficiencies for the 79-d monitoring period for each of the water quality parameters. Statistical analysis was conducted using SPSS 18.0 software. The statistical results were presented in the following form: (ANOVA; $F_{0.95}$ (d.f.; dn); P) where $F_{0.95} = 95\%$ confidence limit; d.f.: degrees of freedom; dn = sample size;

$p > 0.05$ in the related sections of the results and discussion chapter.

3. Results and discussion

3.1. COD removal

Organic matter in wastewater content can be determined as a required amount of oxygen for their chemical oxidation. The required oxygen for aerobic degradation can be provided by diffusion, convection, and oxygen leakage from the macrophyte roots into the rhizosphere [47]. Organic carbon in wetlands can be removed by volatilization, photochemical oxidation, sedimentation, sorption, and biodegradation processes. Organic pollutants are absorbed onto particles flowing into the wetlands which settle out in the quiescent water and are then broken down by the microbiota in the sediment layer. Organic compounds are broken down by the microbiota through fermentation and aerobic/anaerobic respiration, and they are later utilized as a source of energy or are assimilated into biomass. The uptake of organic matter by the macrophytes is negligible when compared to biological degradation [48].

The system fed with an average of 250 mg/L diluted synthetic indigo wastewater. The average effluent concentrations for R1, R2, and R3 were 95.12 ± 14.02, 135.50 ± 26.63, and 152.13 ± 18.07 mg/L, respectively. The average percent removal efficiencies were 61.95 ± 5.61% in reactor R1, 45.80 ± 19.65% in reactor R2, and 39.15 ± 7.23% in reactor R3 (Fig. 3). Statistically significant differences were identified between influent and effluent for each of the three reactor systems (one-way ANOVA; $F_{0.95}(2,75) = 52.33$, $p < \alpha$, $\alpha = 0.05$). After the 91st operation period, there was a statistically meaningful difference between R1 and R2 of 18% and a 23% difference between R1 and R3 ($\alpha < 0.05$). According to the results, plants were capable of COD removal and indigo dye was biodegraded in the first week of the operation. Approximately 70% COD removal by *C. indica* and 55% removal by *T. angustifolia* were observed.

Synthetic dyes that have azo, antraquinon and indigo structures are resistant to microbial biodegradation, so they cannot be removed conventional aerobic methods [49]. In textile industry effluents, COD/BOD_5 ratio is 3–4, which points out the recalcitrance of wastewater effluent [29]. The presence of stable compounds (in the structure of indigo) causes the decrease of COD removal [50]. Plants can uptake and biodegrade polar and ionic structural compounds. Hence, there was a 4% difference in R2 reactor compared to R1 after 8 d which indicates the biodegradation of indigo dye by plants. Fig. 4 presents evidence for transportation of indigo dye from plant roots to leaves. In addition, although influent pH value was 7.95, effluent pH values were 8.19 ± 0.17, 7.91 ± 0.24, and 7.73 ± 0.23 for R1, R2, and R3 reactors, respectively (Fig. 5).

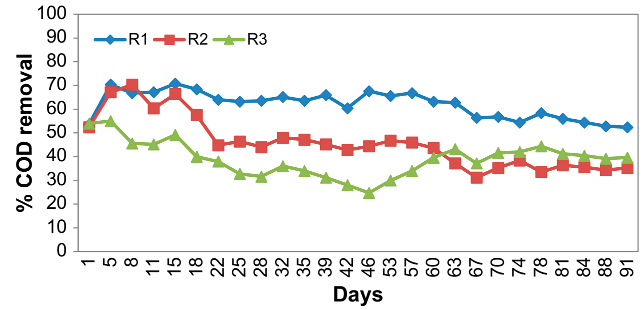

Fig. 3. The COD removal efficiency (%) of the constructed wetland systems (R1: Control, R2: C. *indica*, R3: *T. angustifolia*).

Maximum COD removal was 55% on the 5th day in reactor R3; there was a 15% difference between R2 and R3 reactors (Fig. 3). The COD removal efficiency in reactor R3 was lower than in R2 (Fig. 2); however, although COD removal efficiency in reactor R3 decreased from the 1st to 46th day, it increased after the 46th d. During the operation period, *C. indica*'s root length increased by 27.3%, whereas *T. angustifolia*'s increased by 42.1%. Thus, *T. angustifolia*' root length was greater than *C. indica*'s and *T. angustifolia*'s roots were composed of litter that continuously caused an increase COD concentration due to organic matter addition [51]. Furthermore, the biological reactions are assumed to be due to microbial activity through biofilm formation on the bed material. The low COD removal rates during the 46 d were probably due to the formation of active microorganisms [52].

In reactor R1, a maximum 70% and minimum 52% of COD removal was obtained during the 91st d. Mbuligwe [13] stated that control reactor had a significant role in removal of pollutants in constructed wetland systems. The reason is that constructed wetland systems act as a filter and sedimentation unit and contribute to biological and physical–chemical treatment mechanisms [53–55]. All reactors in this study included 33.33% zeolite media.

Zeolites (clinoptilolite) are materials that are frequently used in various types of wastewaters owing to their large pore size allowing the transition of large particles such as bacteria and organic molecules by adsorption and retention [56]. Clinoptilolite is a natural zeolite filling material with high ion exchange. The

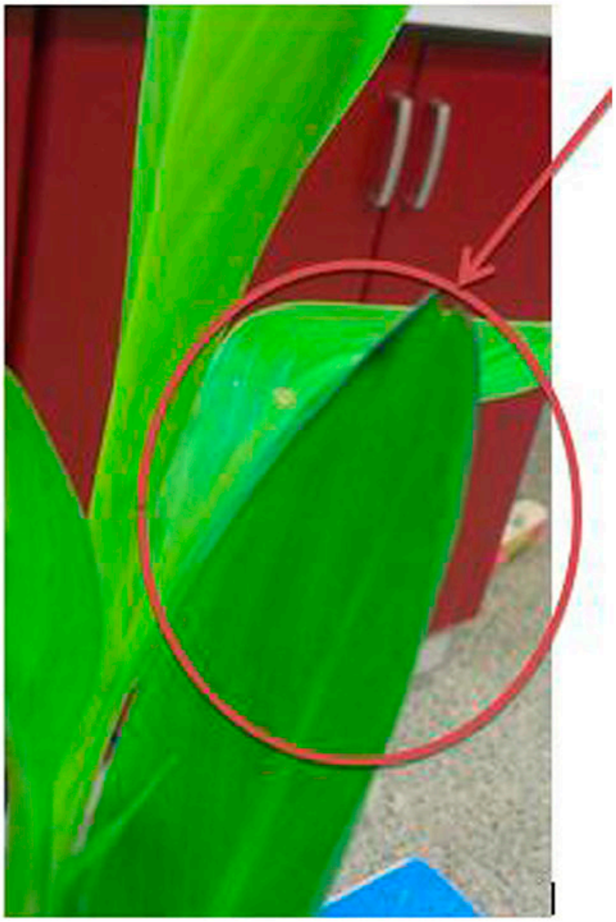

Fig. 4. Change in C. *indica* plant after 8th day operation.

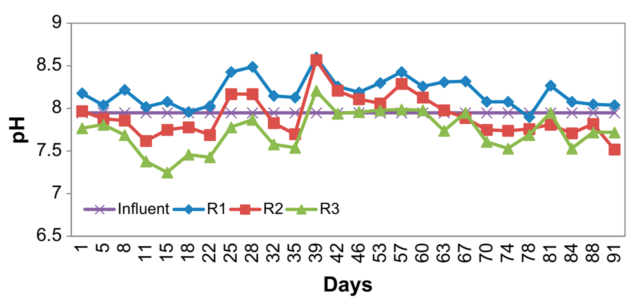

Fig. 5. pH change of the constructed wetland systems (R1: Control, R2: *C. indica*, R3: *T. angustifolia*).

high porosity of zeolite (45–50%) enables the growth of microorganisms [52]. The zeolite obtained from Manisa-Gördes had 39 m^2/g single-point surface area and 1.5–1.9 meq/g cation exchange capacity, and it is a mineral mixture with high levels of Fe and Al (Table 3). Reactor R1 also included zeolite media which could probably provide high COD removal efficiency as shown in Fig. 3.

When comparing the first and 25th days of the operation period, COD removal decreased by 22% for R3 and by 15% for R2. In reactor R1, COD removal varied between 52 and 70% during the 91st d and was stable during the last operation weeks. So, there were organic compound releasing by planted reactors. Meng et al. [57] stated in their studies that plant deaths provide dissolved materials into the environment due to kept continuity of heterotrophic bacterial activities. Crites and Tchobanoglous [55] suggested that dead plant roots could be transformed to nutrients and organic compounds and mixed into the water as additional COD in the effluent, leading to a decrease of removal efficiency.

All reactors stabilized after 63 d and were shut down at the end of 91st d to prevent higher material and energy consumption (Fig. 3).

Conceição et al. [58] treated synthetic textile wastewater including indigo blue dye using a UASB reactor system followed by pottery clay adsorption. According to their study, 81.2% COD removal was achieved. In the study by Sponza et al. [42], acclimation was carried out in two cultures with 30, 60, and 100 mg/L indigo. Forty-five percent of COD removal was observed at 13- and 20-d sludge incubation period. Moreover, Manu and Chaudhari [38] obtained 90% COD removal with an anaerobic semi-continuous reactor. A redox potential of over 100 mV reflects an aerobic medium while lower than −100 mV reflects an anaerobic medium [59]. In our study, all ORP values of all reactors were negative, indicating anoxic operation (Fig. 6).

In recent studies, the best COD removal in wastewater including indigo dye has been achieved by anaerobic processes. As shown in Fig. 6, the control reactor (R1) was close to anaerobic conditions, which led to high removal efficiency in R1.

Zero percentage COD removal was achieved by activated carbon adsorption by Charmagne and Caste [29]. Seven percentage of COD removal efficiency was obtained by a chemical oxidation method using O_3 [32]. Unlu et al. [30] observed 29% COD removal by a microfiltration process and 97% COD removal by nanofiltration after pre-treatment of microfiltration. However, these processes are considerably high cost. Other treatment methods and their drawbacks are stated in Table 4 in detail. A vertical-flow constructed wetland system is basic, easy to assemble, ecologically friendly, and laboratory-scaled; the system removed a maximum of 70% COD at the end of 91st d.

Table 3
Properties of zeolite [50]

Mineral compounds, (volume/volume) (%)	Chemical compounds (volume/volume) (%)	Physical properties
Clinoptilolite 88–95	SiO2 65–72	Porosity 45–50%
Montmorillonite 2–5	Fe_2O_3 0.8–1.9	Hardness 2–3 Ω
Muskovite 0–3	MnO 0–0.08	Surface area 39 m/g
Felspate 3–5	Al_2O_3 10–12	Pore radius 4 Aº
Cristobalite 0–2	MgO 0.9–1.2	pH 7.0–8.0
	CaO 2.5–3.7	
	Na_2O 0.3–0.65	
	K_2O 2.3–3.5	
	TiO_2 0–0.1	
	SiO_2/Al_2O_3 5.4–6.0	

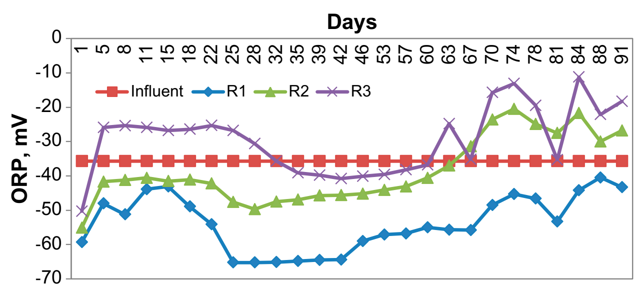

Fig. 6. ORP change of the constructed wetland systems (R1: Control, R2: *C. indica*, R3: *T. angustifolia*).

3.2. Changes in EC

Average inlet EC values in the study were 455 μ/cm, and at the outlet of R1, R2, and R3 reactors, the values were 517.31 ± 45.83, 538.80 ± 51.63, and 658.50 ± 82.77 μs/cm, respectively (one-way ANOVA; $F_{0.95}(3,100) = 62.44$, $p < \alpha$, $\alpha = 0.05$) (Fig. 7). After the 91-d operation periods, the effluent EC values for all reactors were greater than that of the influent EC value. This indicates ion release into the environment for all systems.

Mashauri et al. [60] stated in their study that influent EC value was lower than effluent EC value in a constructed wetland system treating domestic wastewater. This may have indicated the release of nutrient compounds into the wastewater due to plant death, causing an increase of dissolved ions in media. The reason for the high EC value in the system was high ion concentration in the effluent. Increment in the unplanted control reactor could have been due to the release of ions into media by desorption of bedding materials [61].

3.3. Color removal

The maximum wavelength of commercial indigo dye was determined as 613 nm. As a result of measurements taken within that wavelength, percentage color removals in reactors R1, R2, and R3 were 97.10 ± 1.97%, 90.18 ± 5.31%, and 90.15 ± 3.55%, respectively (Fig. 8). There was a statistically significant difference between influent and effluent color removal percentages (one-way ANOVA; $F_{0.95}(2,75) = 26.84$, $p < \alpha$, $\alpha = 0.05$).

In the system, reactor R1 (unplanted) achieved more color removal than the two planted reactors ($p < \alpha$, $\alpha = 0.05$). This is likely due to indigo dye settling and attaching onto plants' roots due its low water solubility. Sediment indigo particles were mixed in the water solution by an intermittent feeding system over time, causing an increase of the color amount. Also, comparatively high color removal in R1 reactor (97%) could have been derived from the intermittent feeding of all reactors and from the use of the same type and amount of filling materials in all reactors, providing an opportunity for biofilm formation in all reactors.

Dogan and Turkdemir [33] treated vat indigo textile dye by an electrochemical oxidation process in their study. After 16 min at pH 2, 100% color removal was investigated using a Pt cage as anode and Pt foil as cathode. In the study by Sponza et al. [42], acclimation was carried out in two cultures at 30, 60, and 100 mg/L indigo. Hundred percentage of color removal efficiency was observed at 13 and 20 d of the sludge incubation period.

García-Morales et al. [32] investigated the color removal efficiency of denim-dyeing facility effluent by electrocoagulation and ozone oxidation. According to their results, 33% of blue color removal was obtained by only ozone oxidation while integration of ozone oxidation and electrocoagulation yielded 84% blue color removal. In aerobic treatment systems, 40–50% of color removal was achieved by adsorption and biodegradation methods [42]. However, these techniques are unfavorable because they lead to high

Table 4
Alternative indigo dye treatment methods and their drawbacks in the literature

Treatment methods in indigo dyeing industry	Drawbacks of treatment methods	Ref.
Biological methods (using of microorganisms, fungi, and enzymes for the degradation and decolorization of dyes	Limited color removal due to toxic nature of some dyes and high salt concentration, pre-treatment mandatory, high COD removal but low decolorization due to chemical stability and microbial attack of dyes, impractical to maintain pure cultures on large scale in actual field conditions	[9,10,40,67–69]
Physical adsorption on activated carbon	Technically easy but highly expensive waste disposal; low COD (0%) and color removal (10%) in vat dyes require regeneration	[29]
Advanced oxidation process with ozone, UV irradiation, Fenton, photocatalytic degradation	Very high cost, low COD, and color removal	[27,32,62–64]
Coagulation and flocculation processes with ferric or aluminum salts or lime	Producing large amounts of wastes, high chemical cost, additional treatment necessity for produced sludge, and higher performance in an integrated systems	[11,43]
Membrane filtration (ultra-micro-nano filtration) methods	Low mass dyes can pass through the membranes, high operation cost, membrane fouling problems, phase change of pollutants	[30,31,65,66]
Electrocoagulation, electroflocculation, electrochemical precipitation	Energy intensive	[5,33,70,71]
Anaerobic degradation by UASB reactors	High economic cost and high sludge production	[42,58]
Aerobic decolorization of the indigo by bioreactors	Hardly degradation of dyes by microorganisms in aerobic environment and low color removal	[41,49]

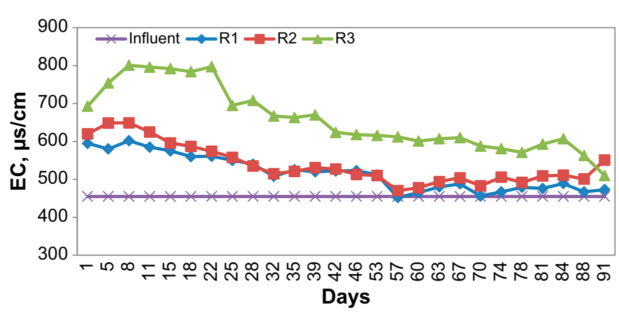

Fig. 7. EC change of the constructed wetland systems (R1: Control, R2: *C. indica*, R3: *T. angustifolia*).

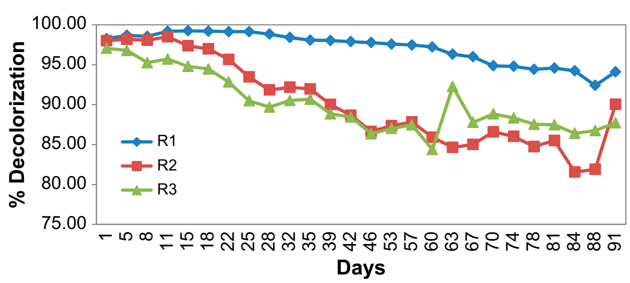

Fig. 8. Decolorization (%) of the constructed wetland systems (R1: Control, R2: *C. indica*, R3: *T. angustifolia*).

sludge production, high-cost operation and chemical requirements, and poor color removal. Color and organic material removal methods which are currently used in the indigo dyeing industry and the drawbacks of each are summarized in Table 4.

Although constructed wetland systems are slow treatment systems, they are simple, low-cost, low energy dependent, green technologies which provide same or better amount of color removal than traditional dye removal systems. Furthermore, in terms of economic considerations, constructed wetlands contribute to natural and local economies by providing water pollution control, creating recreational areas and flood protection. Sludge formation, maintenance, and operation costs are quite low in contrast to alternative treatment methods for textile dyes. In this treatment method, microorganisms and plants play significant roles in color removal from dye-rich wastewater in the following ways: (1) First of all, rhizo-deposition releases 10% of the photosynthetic carbon, leading to increased growth of microorganisms in wastewater, (2) the roots' exudates contain enzymes such as lignin peroxidase, manganese dependent peroxidase, and laccase, which have all been stated to stimulate dye decolorization, (3) oxygen is pumped into the rhizosphere by plant roots and forms the aerobic–anoxic–anaerobic microenvironment that is necessary for the biodegradation of dyes, and (4) dye is adsorbed from wastewater by plants, which could decolorize the water and increase the contact between microorganisms and the azo dyes [72]. In this study, plants were thought to have increased the contact of dye with the microorganisms by adsorbing the dye in wastewater. However, due to low solubility of indigo in water, dye particles could have settled to the filling media which could possibly clog the plant roots.

3.4. Scanning electron microscopy (SEM)

At the end of the 91st day, all systems were shut down and a sample was obtained from the middle layer of zeolite in order to observe the biofilm layer. Since oxygen is necessary for microorganism development and it reaches the zeolite layer last via plant roots, SEM images were obtained from the zeolite layer. Scanning electron microscope images were obtained using a JEOL JSM-6390LV model scanning electron microscope. Fig. 9 shows the empty and biofilm-covered states of zeolite. It demonstrates the coating of the zeolite pores with a dense layer of biofilm. As shown in Fig. 9(C) and (D), the existence of plants in reactor systems could display similar effects on microbial development. As clearly shown in Fig. 9, further intensive biofilm formation was observed in reactor R1 (B). COD removal results that were mentioned above were supported by the SEM images.

3.5. FTIR analysis

FTIR analysis was conducted to observe possible structural changes in the range of 400–4,000 cm^{-1} for commercial indigo dye, obtained from Realkom Textile Factory in Düzce, Turkey, and in constructed wetlands systems during the operational period from day 1 to day 91 of the study. Fig. 10 displays FTIR images for commercial indigo dye before the removal and the changes in R1, R2, and R3 reactors after the removal.

After 91st d, some variations were investigated in effluent FTIR spectra of the indigo dye at the end of constructed wetland operation. A C=O stretching band was observed in R1 and R2 reactors at 1,735 cm^{-1}. Broad O–H stretching was observed at 3,361.93 cm^{-1}

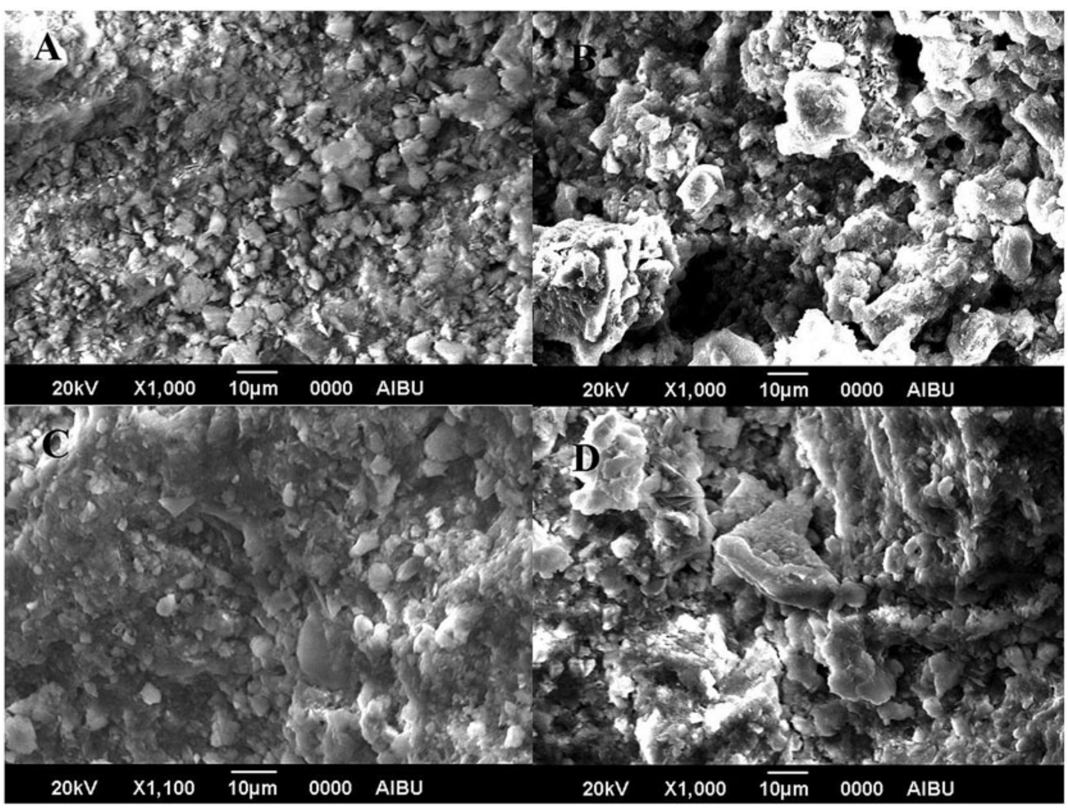

Fig. 9. (A) Scanning electron micrographs of support particle X 1,000, (B) zeolite media of Control Reactor (R1) X 1,000, (C) C. *indica* Reactor (R2) X 1,000 and (D) *T. angustifolia* Reactor (R3) X 1,000.

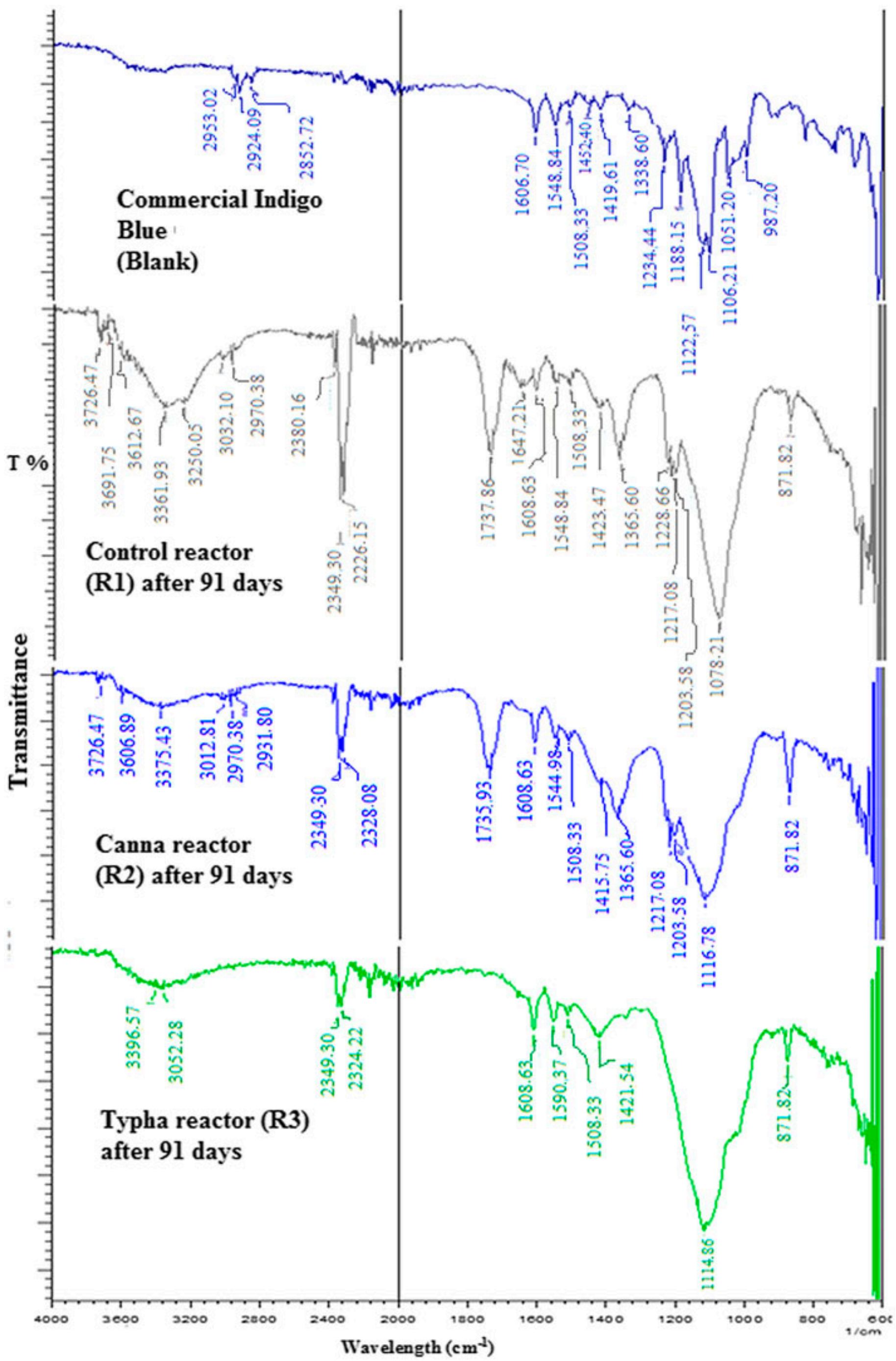

Fig. 10. FTIR analysis of commercial indigo dye before and after experiment.

in R1, 3,375.43 cm^{-1} in R2, and 3,396.57 cm^{-1} in R3. Usually, the appearance of the OH band is considered as indicative of the presence of a hydrated compound whose OH is tightly bound to the molecular structure of the compound [73]. There was a sharper stretching in the control reactor than in the planted reactors (Fig. 8). A sharp secondary amide (N–H) stretching was observed due to molecular conjunction of indigo in R1 at 2,349.30 cm^{-1}. For R2 and R3, amide stretching was obtained at 2,328.08 and 2,324.22 cm^{-1}. However, more studies are needed to quantify each effect (especially the effects of wetland plants on the variation of FTIR spectra) separately and to optimize the operating conditions so as to obtain higher removal efficiencies.

4. Conclusion

In this study, synthetic textile wastewater including commercial indigo dye was treated by a new constructed plant system and color and COD removal efficiencies were observed. At the end of the 91-d operation period, maximum 62% of COD and 97% of color removal ($p < \alpha$) were obtained. Indigo dye was chosen for treatment in the study due to its extensive usage in denim textile factories and due to the color concerns for indigo. Although all reactors contained the same amount of zeolite media, the control reactor exhibited the highest COD and color removal by microbial anoxic degradation, adsorption onto bedding materials, and sedimentation processes. Dead plant litter and roots and particulate dye sediments also caused decreases of COD and color removal during operation periods. EC, pH, and FTIR results demonstrated indigo dye degradation. The study showed that the vertical-flow constructed wetland model can compete with other traditional treatment systems for removal of organic–inorganic pollutants and color.

Acknowledgment

The support for this research was provided by funding from Abant Izzet Baysal University (Bolu-Turkey). [Project Number = 2013.09.04.623].

References

[1] T.G. Bulc, A. Ojstršek, The use of constructed wetland for dye-rich textile wastewater treatment, J. Hazard. Mater. 155(1–2) (2008) 76–82.
[2] T.N. Ramesh, D.V. Kirana, A. Ashwini, T.R. Manasa, Calcium hydroxide as low cost adsorbent for the effective removal of indigo carmine dye in water, J. Saudi Chem. Soc. (in press), doi: 10.1016/j.jscs.2015.03.001.
[3] Y.H. Lee, S.G. Pavlostathis, Decolorization and toxicity of reactive anthraquinone textile dyes under methanogenic conditions, Water Res. 38 (2004) 1838–1852.
[4] A. Ojstršek, D. Fakin, D. Vrhovšek, Residual dyebath purification using a system of constructed wetland, Dyes Pigm. 74 (2007) 503–507.
[5] C. Cameselle, M. Pazos, M.A. Sanromán, Selection of an electrolyte to enhance the electrochemical decolorisation of indigo. Optimisation and scale-up, Chemosphere 60 (2005) 1080–1086.
[6] B. Manu, S. Chaudhari, Anaerobic decolorisation of simulated textile wastewater containing azo dyes, Bioresour. Technol. 82 (2002) 225–231.
[7] G.M. Shaul, T.J. Holdsworth, C.R. Dempsey, K.A. Dostal, Fate of water-soluble azo dyes in the activated-sludge process, Chemosphere 22 (1991) 107–119.
[8] P.K. Gill, D.S. Arora, M. Chander, Biodecolourization of azo and triphenylmethane dyes by *Dichomitus squalens* and *Phlebia spp*, J. Ind. Microbiol. Biotechnol. 28 (2002) 201–203.
[9] A. Bes-Piá, M.I. Iborra-Clar, A. Iborra-Clar, J.A. Mendoza-Roca, B. Cuartas-Uribe, M.I. Alcaina-Miranda, Nanofiltration of textile industry wastewater using a physicochemical process as a pre-treatment, Desalination 178 (2005) 343–349.
[10] T. Robinson, G. McMullan, R. Marchant, P. Nigam, Remediation of dyes in textile effluent: A critical review on current treatment technologies with a proposed alternative, Bioresour. Technol. 77 (2001) 247–255.
[11] M. Sala, M.C. Gutiérrez-Bouzán, Electrochemical techniques in textile processes and wastewater treatment, Int. J. Photoenergy 2012 (2012) 1–12.
[12] M.A. Rauf, S. Salman Ashraf, Survey of recent trends in biochemically assisted degradation of dyes, Chem. Eng. J 209 (2012) 520–530.
[13] S.E. Mbuligwe, Comparative treatment of dye-rich wastewater in engineered wetland systems (EWSs) vegetated with different plants, Water Res. 39 (2005) 271–280.
[14] S.A. Ong, K. Uchiyama, D. Inadama, K. Yamagiwa, Simultaneous removal of color, organic compounds and nutrients in azo dye-containing wastewater using up-flow constructed wetland, J. Hazard. Mater. 165 (2009) 696–703.
[15] L.C. Davies, I.S. Pedro, J.M. Novais, S. Martins-Dias, Aerobic degradation of acid orange 7 in a vertical-flow constructed wetland, Water Res. 40 (2006) 2055–2063.
[16] A.K. Yadav, S. Jena, B.C. Acharya, B.K. Mishra, Removal of azo dye in innovative constructed wetlands: Influence of iron scrap and sulfate reducing bacterial enrichment, Ecol. Eng. 49 (2012) 53–58.
[17] A. Yalcuk, The macro nutrient removal efficiencies of a vertical flow constructed wetland fed with demineralized cheese whey powder solution, Int. J. Phytorem. 14 (2012) 114–127.
[18] M.Y. Sultana, A.K.Md.M.B. Chowdhury, M.K. Michailides, C.S. Akratos, A.G. Tekerlekopoulou, D.V. Vayenas, Integrated Cr(VI) removal using constructed wetlands and composting, J. Hazard. Mater. 281 (2015) 106–113.
[19] J. Vymazal, M. Greenway, K. Tonderski, H. Brix, U. Mander, Constructed Wetlands for Wastewater Treatment. Series in Ecological Studies, Springer-Verlag, Berlin, Heidelberg, 190 (2006).
[20] V.A. Tsihrintzis, C.S. Akratos, G.D. Gikas, D. Karamouzis, A.N. Angelakis, Performance and cost comparison of a FWS and aVSF constructed wetland system, Environ. Technol. 28(6) (2007) 621–628.
[21] Y. Uysal, F. Taner, The effect of cadmium ions on the growth rate of the freshwater macrophyte duckweed lemna minor, Ekoloji 16(62) (2007) 9–15.
[22] H.C. Tee, C.E. Seng, A.Md. Noor, P.E. Lim, Performance comparison of constructed wetlands with gravel- and rice husk-based media for phenol and nitrogen removal, Sci. Total Environ. 407 (2009) 3563–3571.

[23] A. Vuorema, Reduction and Analysis Methods of Indigo, Turun Tliopistan Julkaisuja, Annalaes Universitatis Turkuensis, Turku-Finland, 2008, pp. 1–72. Available from <https://www.doria.fi/bitstream/handle/10024/42825/AI388%20Vuorema.pdf> (Accessed: 06. 04. 2015).
[24] M. Irimia-Vladu, E.D. Głowacki, P.A. Troshin, G. Schwabegger, L. Leonat, D.K. Susarova, O. Krystal, M. Ullah, Y. Kanbur, M.A. Bodea, V.F. Razumov, H. Sitter, S. Bauer, N.S. Sariciftci, Indigo - A natural pigment for high performance ambipolar organic field effect transistors and circuits, Adv. Mater. 24(3) (2012) 375–380.
[25] M. Vedrenne, R. Vasquez-Medrano, D. Prato-Garcia, B.A. Frontana-Uribe, M. Hernandez-Esparza, J.M. de Andrés, A ferrous oxalate mediated photo-Fenton system: Toward an increased biodegradability of indigo dyed wastewaters, J. Hazard. Mater. 243 (2012) 292–301.
[26] K. Harazono, K. Nakamura, Decolorization of mixtures of different reactive textile dyes by the white-rot basidiomycete *Phanerochaete sordida* and inhibitory effect of polyvinyl alcohol, Chemosphere 59 (2005) 63–68.
[27] R. Qu, B. Xu, L. Meng, L. Wang, Z. Wang, Ozonation of indigo enhanced by carboxylated carbon nanotubes: Performance optimization, degradation products, reaction mechanism and toxicity evaluation, Water Res. 68 (2015) 316–327.
[28] S. Hammami, M.A. Oturan, N. Oturan, N. Bellakhal, M. Dachraoui, Comparative mineralization of textile dye indigo by photo-Fenton process and anodic oxidation using boron-doped diamond anode, Desalin. Water Treat. 45 (2012) 297–304.
[29] O. Charmagne, C. Caste, Color Removal from Textile Plant Effluents, American Dyestuff Reporter, Degremont S.A. Cedex, 1996.
[30] M. Unlu, H. Yukseler, U. Yetis, Indigo dyeing wastewater reclamation by membrane-based filtration and coagulation processes, Desalination 240 (2009) 178–185.
[31] N. Uzal, L. Yilmaz, U. Yetis, Microfiltration: A pretreatment alternative for indigo dyeing textile wastewater, Desalination 199 (2006) 515–517.
[32] M.A. García-Morales, G. Roa-Morales, C. Barrera-Díaz, P. Balderas-Hernández, B. Bilyeu, An integrated electrocoagulation and ozonation process for removal of indigo carmine dye from denim processing effluent, Sustainable Environ. Res. 23(4) (2013) 241–245.
[33] D. Dogan, H. Turkdemir, Electrochemical treatment of actual textile indigo dye effluent, Pol. J. Environ. Stud. 21(5) (2012) 1185–1190.
[34] M.A. Sanromán, M. Pazos, M.T. Ricart, C. Cameselle, Decolourisation of textile indigo dye by DC electric current, Eng. Geol. 77 (2005) 253–261.
[35] T. Bechtold, E. Burtscher, A. Turcanu, O. Bobleter, Indirect electrochemical reduction of dispersed indigo dyestuff, J. Electrochem. Soc. 143(8) (1996) 2411–2416.
[36] E. Butron, M.E. Juarez, M. Solis, M. Teutli, I. Gonzalez, J.L. Nava, Electrochemical incineration of indigo textile dye in filter-press-type FM01-LC electrochemical cell using BDD electrodes, Electrochim. Acta. 52(24) (2007) 6888–6894.
[37] D.S.L. Balan, R.T.R. Monteiro, Decolorization of textile indigo dye by ligninolytic fungi, J. Biotechnol. 89 (2001) 141–145.
[38] B. Manu, S. Chaudhari, Decolorization of indigo and azo dyes in semicontinuous reactors with long hydraulic retention time, Process Biochem. 38 (2003) 1213–1221.
[39] S.E. Ruíz-Balaguera, L. Rincón-Chisino, E. Herrera-Sastoque, L. Castillo-Carvajal, B. Barragán-Huerta, A.M. Pedroza-Rodríguez, Indigo removal by *Pleurotus ostreatus* under different nutritional conditions, J. Biotechnol. 150(Suppl. 1) (2010) 228.
[40] C. Tian, R. Tian, Y. Zhou, Q. Chen, H. Cheng, Decolorization of indigo dye and indigo dye-containing textile effluent by *Ganoderma weberianum*, Afr. J. Microbiol. Res. 7(11) (2013) 941–947.
[41] E. Khelifi, H. Gannoun, Y. Touhami, H. Bouallagui, M. Hamdi, Aerobic decolourization of the indigo dye-containing textile wastewater using continuous combined bioreactors, J. Hazard. Mater. 152 (2008) 683–689.
[42] D. Sponza, M. Işık, H. Atalay, İndigo boyar maddelerinin anaerobik arıtılabilirliklerinin incelenmesi, DEÜ Müh. Fak. Fen Müh (The investigation of anaerobictreability of indigo dyes, DEU faculty of engineering). Dergisi 2(3) (2000) 23–34.
[43] B. Manu, Physico-chemical treatment of indigo dye wastewater, Color. Technol. 123 (2007) 197–202.
[44] O. Phewnil, K. Chunkao, T. Pattamapitton, A. Intaraksa, O. Chueawong, C. Chantrasoon, T. Boonprakong, Choosing aquatic plant species for high wastewater treatment efficiency through small wetland, Mod. Appl. Sci. 8(4) (2014) 187–194.
[45] G. Sharma, P. Brighu, U. Brighu, Performance analysis of vertical up-flow constructed wetlands for secondary treated effluent, Procedia APCBEE 10 (2014) 110–114.
[46] American Public Health Association (APHA), Standard Methods for the Examination of Waste and Wastewater, twenty-first ed., APHA, Washington, DC, 2005.
[47] A. Korkusuz, M. Beklioglu, G. Demirer, Comparison of the treatment performances of blast furnace slag-based and gravel- based vertical flow wetlands operated identically, for domestic wastewater treatment in Turkey, Ecol. Eng. 24 (2005) 187–200.
[48] ITRC, Technical and regulatory guidance document for constructed wetlands (2003). Available from: <www.afcee.brooks.af.mil/products/techtrans/download/WTLND-1.pdf> (Accessed 2007).
[49] H.G. Kulla, Aerobic bacterial degradation of azo dyes, FEMS symp. 12 (1981) 387–399.
[50] A. Yalcuk, Removal of phenol from olive mill wastewater in constructed wetlands using different bedding media, Ekoloji 20(80) (2011) 1–5.
[51] A. Yalcuk, Use of Constructed Wetlands for Treatment of Landfill Leachate, PhD Thesis, Hacettepe University, Natural Science, Ankara, (2007).
[52] A. Yalcuk, A. Ugurlu, Comparison of horizontal and vertical constructed wetland systems for landfill leachate treatment, Bioresour. Technol. 100 (2009) 2521–2526.
[53] R.H. Kadlec, R.L. Knight, Treatment Wetlands, Lewis Publishers, Boca Raton, Florida, 1996.

[54] P.F. Cooper, G.D. Job, M.B. Green, R.B. Shutes, Reed beds and Constructed Wetlands for Wastewater Treatment, WRc Swindon, Wiltshire, 1996.
[55] R. Crites, G. Tchobanoglous, Small and Decentralized Wastewater Management Systems, McGraw-Hill, Boston, 1998.
[56] A. Pınar, A. Ugurlu, Ammonium removal from leachate by Turkish clinoptilolite: Effect of competing ions, 9th International Waste Management and Landfill Symposium, Cagliari, Sardinia, Italy, 2003.
[57] P. Meng, H. Pei, W. Hu, Y. Shao, Z. Li, How to increase microbial degradation in constructed wetlands: Influencing factors and improvement measures, Bioresour. Technol. 157 (2014) 316–326.
[58] V. Conceição, F. Bentes Freire, K. Querne de Carvalho, Treatment of textile effluent containing indigo blue dye by a UASB reactor coupled with pottery clay adsorption, Acta Sci. Technol. 35(1) (2013) 53–58.
[59] S.S. Suthersan, Natural and Enhanced Remediation Systems, Acradis, Lewis Publisher, Washington, DC, 2002.
[60] D.A. Mashauri, D.M.M. Mulungu, B.S. Abdulhussein, Constructed wetland at the University of Dar es Salaam, Water Res. 34(4) (2000) 1135–1144.
[61] A. Yalcuk, N.B. Pakdil, S.Y. Turan, Performance evaluation on the treatment of olive mill waste water in vertical subsurface flow constructed wetlands, Desalination 262 (2010) 209–214.
[62] M.T.F. Tabrizi, D. Glasser, D. Hildebrandt, Wastewater treatment of reactive dyestuffs by ozonation in a semi-batch reactor, Chem. Eng. J. 166 (2012) 662–668.
[63] S. Wang, A comparative study of Fenton and Fenton-like reaction kinetics in decolourisation of wastewater, Dyes Pigm. 76(3) (2008) 714–720.
[64] N.M. Mahmoodi, M. Arami, N.Y. Limaee, N.S. Tabrizi, Decolorization and aromatic ring degradation kinetics of Direct Red 80 by UV oxidation in the presence of hydrogen peroxide utilizing TiO2 as a photocatalyst, Chem. Eng. J. 112(1–3) (2005) 191–196.
[65] Y. Zheng, S. Yu, S. Shuai, Q. Zhou, Q. Cheng, M. Liu, C. Gao, Color removal and COD reduction of biologically treated textile effluent through submerged filtration using hollow fiber nanofiltration membrane, Desalination 314 (2013) 89–95.
[66] N. Tahri, G. Masmoudi, E. Ellouze, A. Jrad, P. Drogui, R. Ben Amar, Coupling microfiltration and nanofiltration processes for the treatment at source of dyeing-containing effluent, J. Clean. Prod. 33 (2012) 226–235.
[67] I. Oller, S. Malato, J.A. Sánchez-Pérez, Combination of advanced oxidation processes and biological treatments for wastewater decontamination—A review, Sci. Total Environ. 409 (2011) 4141–4166.
[68] R. Campos, A. Kandelbauer, K.H. Robra, A. Cavaco-Paulo, G.M. Gübitz, Indigo degradation with purified laccases from *Trametes hirsuta* and *Sclerotium rolfsii*, J. Biotechnol. 89(2–3) (2001) 131–139.
[69] G.S. Nyanhongo, J. Gomes, G.M. Gübitz, R. Zvauya, J. Read, W. Steiner, Decolorization of textile dyes by laccases from a newly isolated strain of *Trametes modesta*, Water Res. 36(6) (2002) 1449–1456.
[70] S.H. Lin, C.F. Peng, Treatment of textile wastewater by electrochemical method, Water Res. 28 (1994) 277–282.
[71] S. Ammar, R. Abdelhedi, C. Flox, C. Arias, E. Brillas, Electrochemical degradation of the dye indigo carmine at boron doped diamond anode for wastewaters remediation, Environ. Chem. Lett. 4 (2006) 229–233.
[72] X. Zhou, X. Xiang, Effect of different plants on azo-dye wastewater bio- decolorization, Procedia Environ. Sci. 18 (2013) 540–546.
[73] A. Trujillo-Ortega, S.A. Martinez Delgadillo, V.X. Mendoza-Escamilla, M. May-Lozano, C. Barrera-Diaz, Modeling the removal of indigo dye from aqueous media in a sonoelectrochemical flow reactor, Int. J. Electrochem. Sci. 8 (2013) 3876–3887.