


Article

Removal of Acid Dyes from Textile Wastewaters Using Fish Scales by Absorption Process

S M Fijul Kabir ^{1,3,*} , Rafael Cueto ², Sreelatha Balamurugan ², Laurel D. Romeo ³, Jenna T. Kuttruff ³, Brian D. Marx ⁴ and Ioan I. Negulescu ³

¹ Department of Textile Engineering, Chemistry and Science, Wilson College of Textiles, North Carolina State University, Raleigh, NC 27695, USA

² Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803, USA; rcueto@lsu.edu (R.C.); latha@lsu.edu (S.B.)

³ Department of Textiles, Apparel Design and Merchandising, Louisiana State University, Baton Rouge, LA 70803, USA; ldromeo@lsu.edu (L.D.R.); jkuttruff@agcenter.lsu.edu (J.T.K.); INegulescu@agcenter.lsu.edu (I.I.N.)

⁴ Department of Experimental Statistics, Louisiana State University, Baton Rouge, LA 70803, USA; bmarx@lsu.edu

* Correspondence: skabir@ncsu.edu; Tel.: +1-22-5284-7920

Received: 27 August 2019; Accepted: 8 October 2019; Published: 14 October 2019



Abstract: Fish scales (FS), a byproduct of the fish processing industry, are often discarded carelessly. In this present study, FS were used as a promising bio-sorbent for the removal of anionic acid dyes (acid red 1 (AR1), acid blue 45 (AB45) and acid yellow 127 (AY127)) from the wastewaters of textile coloration. Here, physiochemical characterizations of the FS were investigated by SEM-EDS, TGA and FI-IR analyses, and dye absorption and removal efficiency were evaluated and optimized considering different process parameters such as concentration of initial dye solution, amount of FS used, contact time, FS size, process temperature, additives, stirring and vacuum. SEM images and EDS elemental analyses showed architectural variation and heterogeneous composition of FS at different places. TGA identified the 50% minerals, 33% organic matters and 17% moisture and volatile components. FI-IR evidenced considerable absorption of acid dyes. Process optimization revealed that additives and fine pulverized FS had significant positive and negative impact on the dye removal efficacy, respectively. Temperature and stirring improved dye removal efficiency, and dye absorption by FS was very fast at the beginning and became almost constant after an hour indicating saturation of absorption. The maximum dye absorptions in scales for AR1, AB45, and AY127 were noted as 1.8, 2.7 and 3.4 mg/g, respectively, and removal percentages were 63.5%, 89.3% and 93%. The effects of the process parameters were consistent across all three acid dyes used in this study. Two-way ANOVA model showed that dye type, process parameters and ‘dye type X process parameters’ interactions had significant effect on the dye removal efficiency.

Keywords: fish scales (FS); anionic dyes; dye absorption and adsorption; collagen; dye removal

1. Introduction

Over two thirds of our planet is covered by water; however, only three percent is freshwater. Pitifully, out of this tiny amount, less than one third is potable, since the remaining portion is locked in the form of glaciers, ice, and snow on the north and south poles [1]. Additionally, two-thirds of the freshwater is withdrawn for industrial purposes such as chemical, agriculture, leather, cosmetic, steel, refinery, cement, and textile processing. The textile industry requires large amounts of water usage along the supply chain from fiber to apparel production. In addition to consuming a significant

portion of available freshwater, the textile industry has come under scrutiny for polluting water with its waste [2]. The wastewaters of textile coloration contain nonbiodegradable synthetic dyes, high oxygen demanding materials, and other noxious chemicals [3]. As a result, when these wastewaters are discharged to the environment without any treatment, it adversely affects all the basic biotic and abiotic components of environment including the hydrosphere, lithosphere and atmosphere [4].

The growing worldwide concern for environmental and human safety has sparked increased interest in how these wastewaters are treated before discharging to the environment. To treat textile wastewaters, methods such as: Physicochemical processes (sorption, reverse osmosis, ion exchange), biological processes (aerobic and anaerobic treatment, microbial reduction, bacterial treatment), nuclear treatments (irradiation by nuclear radiations), acoustical, electrical, and electromagnetic processes (ultrasonic treatment) are available [5]. Among these, the sorption process is now considered as the most effective and economical way to treat textile wastewaters. Among the materials used for textile wastewater treatments by sorption process, fish wastes are mentioned in the recent investigations as a promising, environmentally friendly and economically very affordable solution [5]. Also, instead of careless dumping of fish scales (FS) with no economic value, and creating pungent odor to the environment, using FS as a bio-sorbent may offer mutual management of both fish wastes and textile wastewaters.

In the structure of FS, there exists an organic protein matter called collagen that is mainly responsible for anionic dye absorption. There is also an inorganic mineral substance called hydroxyapatite that is responsible for adsorption [6]. The scales from different types of fishes have been used as adsorbents for anionic dye removal. Recent studies have investigated *Labeo rohita* for reactive dyes, such as reactive red 2 [7] and reactive orange 16 [8]. The *Oreochromis niloticus* has been used for reactive blue 5G [9–11] and the *Leporinus elongatus* for remazol (yellow, blue and red) [12]. Attempts were also made for cationic basic dye, methylene blue dyes, but outcomes were not much encouraging [13].

Studies investigating these adsorption processes have prepared the FS both chemically and/or physically with a view to boosting up adsorption capacity; for instance, researchers have used NaOH [6,12], sodium hypochlorite [10], EDTA [14], high temperature [6,7,10,12,15], and carbonized FS [8,13]. Additionally, in an attempt to obtain maximum specific area of scales to increase more dye accumulation on the surface, powdered scales of different sizes have been used [6,7,10,16]. Regardless of the physical and/or chemical process involved, Eaglen and Pollak [17] noted that some investments for the processes, in terms of money, time, and labor are required. They note that the mineral content (apatites) of the FS is mainly responsible for adsorption of dyes. In contrast, dye removal performance based on absorption is barely found.

In this research, a previously unpublished approach to the use of FS for dye absorption was investigated. FS of black drum (*Pogonias cromis*) have been proposed for the absorption of anionic acid dyes (acid red 1, acid blue 45 and acid yellow 127) without any physical or chemical modification to avoid process complexity and approach an economical solution for dye removal. Acid dyes are one the most frequently used dyes in the dyeing of natural protein fibers (i.e., wool) and synthetic polyamide fibers (i.e., nylon) [18]. The organic content of black drum, collagen, contains different cationic amide derivatives (amide-A, amide-I, and amide-II) [19], which might have strong affinity to the anionic portion of acid dyes and become responsible for dye absorption. Through the present research, the performance of FS of black drum as bio absorbent for acid dye removal without any prior physical and chemical preparation has been assessed.

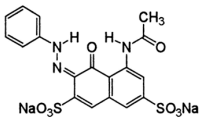
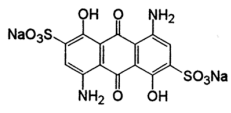
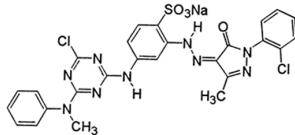
2. Methods

2.1. Materials and Chemicals

The FS along with skins of black drum fishes were donated by Big D, Franklin, LA, USA. The selected water soluble anionic acid dyes (nonbiodegradable) listed in Table 1 were purchased from Crompton & Knowles Corporation (Middlebury, CT, USA), acid (sulfuric acid) from Fischer

Scientific (Hampton, NH, USA) salt (sodium sulfate) from Mallinckrodt (Raleigh, NC, USA), and all other chemicals such as acetone from Sigma-Aldrich (Raleigh, NC, USA).

Table 1. Structures and properties of acid dyes used in this study.

Chemical Structures	Molecular Weights	Wavelength in nm at Maximum Absorption	Molar Coefficients in $M^{-1} cm^{-1}$
 <p>Acid red 1 (AR1)</p>	509.42	506	19,593
 <p>Acid blue 45 (AB45)</p>	474.33	595	7048.4
 <p>Acid yellow 127 (AY127)</p>	803.63	407	8658.8

2.2. Preparation of FS

FS were separated from the skins. They were then washed thoroughly with normal tap water, dried at room temperature, and ground. The ground FS were then fractionated using sieves of different mesh sizes (no. 40, 60, 100, and 200). Rest pulverized FS and the whole pristine FS were also used for absorption purpose.

2.3. Preparation of Dye Solution and Absorption Process

Simulated sample dye solutions (considered as sample wastewater solution) were prepared following the standard wool dyeing recipe: 50, 75, 100, 125, 150 and 200 mg/L dye, 62.5 mg/L salt (Na_2SO_4), and 7.5 mg/L acid (H_2SO_4) [20]. Each time, 20 mL dye wastewater sample was taken in a treatment bath. Afterward, FS were added to the dye solutions, absorption process was applied in a closed system with continuous magnetic stirring. Finally, the dye-scale mixtures were centrifuged for five minutes at 4000 rpm using IEC clinical centrifuge (International Equipment Company, Thermo Scientific IEC, Bellport, NY, USA) to have clear solutions, which were the final solutions after absorption. The effect of the different dyeing and absorption process parameters such as concentration of dyes, amount of the scales used, size of the scales, contact time, temperature, stirring, and vacuum were analyzed. Additionally, to understand the contribution of the presence of additives (acid and salt) in the absorption process, investigations were also conducted without those additives together, and individually. Tables 2 and 3 summarize all the process conditions investigated in this research.

Table 2. Effects of temperature, salt, acid, additives (acid and salt), stirring, and vacuum.

ID for Statistical Analysis	Treatments	Parameter Applied ('√' Mark Means Applied, '-' if Not)					
		Temperature (60° C)	Salt 62.5 mg/L	Acid 7.5 mg/L	Additives (Acid and Salt Together)	Stirring (1000 rpm)	Vacuum for 1.5 h after Absorption Process
Control	Std.	√	√	√	√	√	-
AR1/AB45/AY127 2	No temp	-	√	√	√	√	-
AR1/AB45/AY127 3	No salt	√	-	√	-	√	-
AR1/AB45/AY127 4	No acid	√	√	-	√	√	-
AR1/AB45/AY127 5	No additives	√	-	-	-	√	-
AR1/AB45/AY127 6	No Stirring	√	√	√	√	-	-
AR1/AB45/AY127 7	Vacuum effect	√	√	√	√	√	√
Other parameters: 50 mg/L dye solutions, 1 h process time, 50 g/L large pulverized FS							

Table 3. Effects of initial dye concentration, amount of fish scales (FS) used, scale size and contact time.

Treatment	Treatment Levels	Other Process Conditions
Effect of dye concentration	50, 75, 100, 125, 150 and 200 mg/L	As control in Table 2
Effect of amount of FS used	25, 50, 75 and 100 g/L	
Scale size	Whole scales, large pulverized FS and powdered FS with sieve no. 40 (420 μ), 60 (250 μ), 100 (149 μ), 200 (74 μ)	
Effect of time	0.25, 0.5, 1, 1.5, 2 and 2.5 h	

2.4. Characterization

The physiochemical characterization (SEM-EDS and FT-IR) were performed for both pristine and blue colored FS. Structures and morphology of the scales were unveiled by taking surface pictures at different magnifying levels such as 100×, 1000× and 5000× using scanning electron microscopy (SEM) (JEOL, Tokyo, Japan) in low vacuum condition at 15 kv [10]. Zeiss Lumera light microscope (Carl-Zeiss, Oberkochen, Germany) provided 6.4× image of whole scale of both sides. Energy dispersive X-ray spectroscopy (EDS) (EDAZ AMETEK material analysis division, Mahwah, NJ, USA), coupled with the SEM system was used in order to recognize the elements present in the FS [21]. Before SEM-EDS analysis, samples (colored and uncolored) were coated with a thin gold layer using EMS550X sputter coater (Electron Microscopy Sciences, Hatfield, PA, USA). For thermogravimetric (TGA) analysis, 2.03 mg FS were heated under nitrogen atmosphere at a heating rate of 10 °C/min up to 600 °C using AutoTGA 2950HR V5.4A (Thermal Instruments, Trevose, PA, USA). Fourier transform infrared (FT-IR) spectroscopy analysis was undertaken to disclose functional groups [22] present in the pristine scales, powdered scales, dyes (AB45) and colored scales between 400 and 4000 cm⁻¹ wavenumber using Bruker Alpha and Tensor 27 FT-IR and OPUS software (Billerica, MA, USA).

2.5. Determination of Dye Removal Efficiency

The dye removal efficiency was evaluated using Equation (1), where C_o and C_e are concentrations of dye in mg/L in wastewaters before and after absorption. Also, the amount of absorbed dye in the scales was measured in mg/g at the equilibrium (q_e) using Equation (2) [23].

$$\text{Dye removal (\%)} = (C_o - C_e) \times 100\%/C_o \quad (1)$$

$$q_e = (C_o - C_e)/X_o \quad (2)$$

X_0 is the amount of absorbent used in the absorption process in g/L. The concentrations of wastewaters after dye absorption were calculated using Beer–Lambert law (Equation (3)) and UV-Vis spectroscopy (HP8453, Agilent, Santa Clara, CA, USA) [24].

$$A = \epsilon bc \quad (3)$$

A is the absorbance, ϵ is the calibrated molar coefficient in $M^{-1} \text{ cm}^{-1}$ (obtained values are in Table 1), b is the path length of the cuvette (usually 1 cm), c is the concentration in mole/L [25].

2.6. Statistical Analysis

A two-way analysis of variance (ANOVA) at 5% level of significance using statistical analysis software (SAS) 9.4 was conducted to investigate whether or not there are any significant differences in the removal percentages among different dyes (AR1, AB45 and AY127), among different process parameters (temperature, salt, acid, additives (salt and temperature together) stirring and vacuum) and among ‘dye type X process’ interactions. Types of dyes and process parameters were two main effects, having 3 and 7 levels of fixed treatments, respectively. Based on the ANOVA results, the effects of different process parameters within each dye type were also conducted relative to the standard or control process using Dunnett’s adjustment. Here, we had four replications for the control process and one for the other treatment levels.

3. Result and Discussion

3.1. Characterization

3.1.1. Drum’s Scale Architecture

The 6.4× pictures (Figure 1) of both colored and pristine FS were taken by light microscopy under the same light source, which exhibit exquisite architectural variations in the disk-like cycloid typed scale of black drum [26]. Figure 1A,B show the top (rough) and bottom (smooth) sides of pristine FS, respectively. Although they provide similar visual appearances, they are both chemically and structurally different.

The top side (Figure 1A) containing hard mineral (as per EDS report) has different orientations identified by numbers 1, 2, 3, 4 and 5: (1) The anterior field, the front part, separated by white inflexion lines called radii, has stair like ridges, (2) the focus, the center of the FS, is relatively hard in structure with mosaic patterns, (3) the posterior field embedded to the body of fish is decorated with meander lined tiles, (4) the circuli covering maximum area of the scale is featured with arc type lines, and (5) the lateral field that distinguishes the circuli and the posterior field [26]. The bottom side (Figure 1B) of the scale that comes into contact with the body of the fish is smooth and comprised of mostly a jelly-like organic matter [6]. No structural variation was found even in the highly magnified SEM images of 100×, 1000× and 5000×. As the absorption is a chemical process, the different composition of the top and bottom of the FS resulted in variations of dye absorption. This is also visually evident from the different shades found on the top and bottom of the FS (Figure 1C,D).

3.1.2. SEM and EDS

Figure 2 shows SEM images of both sides of the FS before (A and C) and after (B and D) dye absorption. No significant differences have been perceived between the images, which is in agreement with Uzunoğlu and Özer’s [15] observations. Also, Ribeiro et al. [10] attributed that the untreated FS surfaces are non-porous leading to a poor adsorption, although some cracks are visible in the top side of the scale (Figure 2A,B). Depleting the crack size in the colored top side relative to pristine might imply some dye anchoring. However, the change in the pore size before and after absorption is not as obvious as noted by Zhu et al. [6]. Zhu et al. processed FS with NaOH and applied

high temperature to improve adsorption. In the study, no chemical treatment or external heating were applied to avoid extra cost and process complexity.

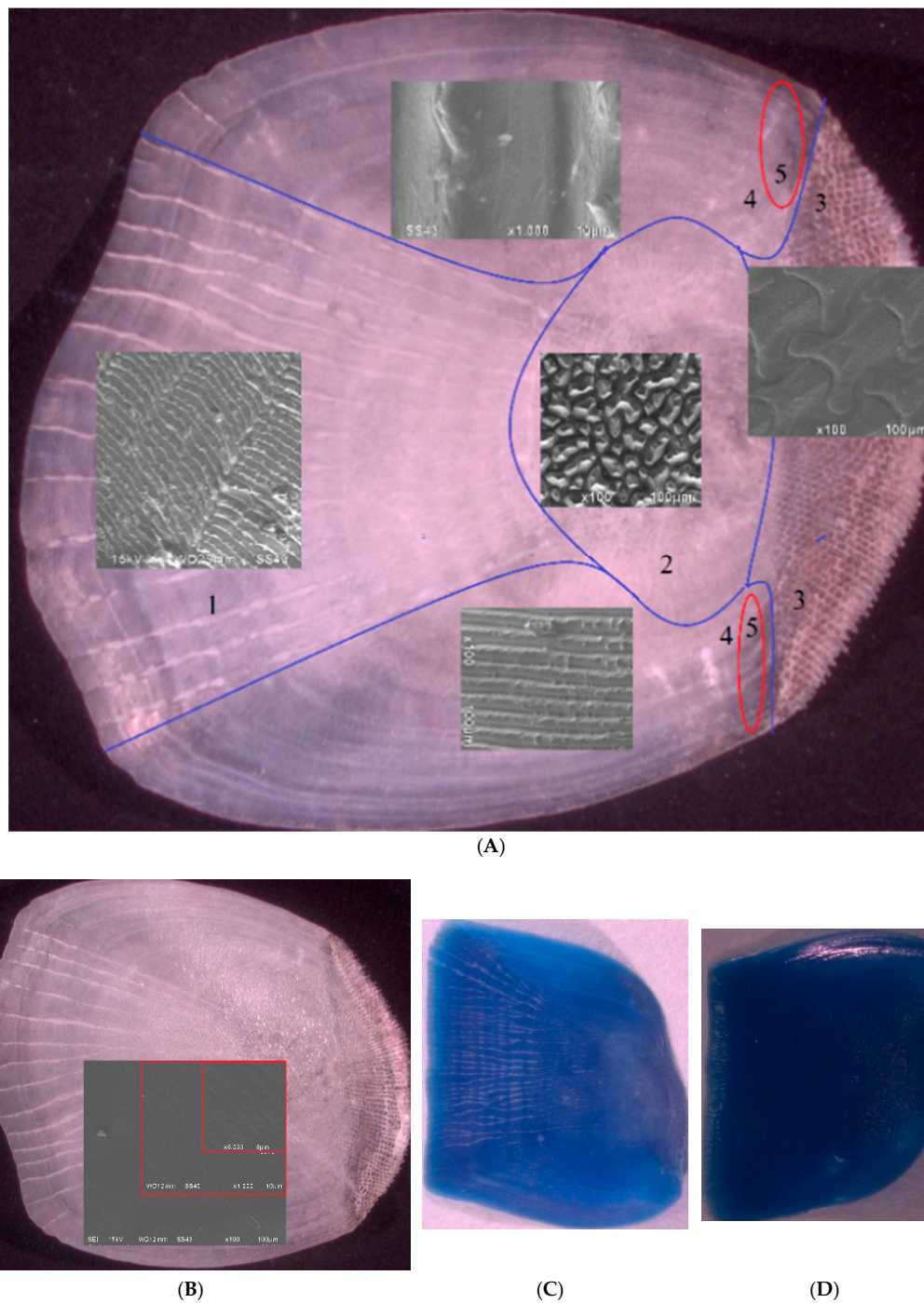


Figure 1. Pristine top surface (A) (1: The anterior field, 2: The focus, 3: The posterior field, 4: The circuli, 5: The lateral fields), bottom surface (B), colored top surface (C) and colored bottom surface (D).

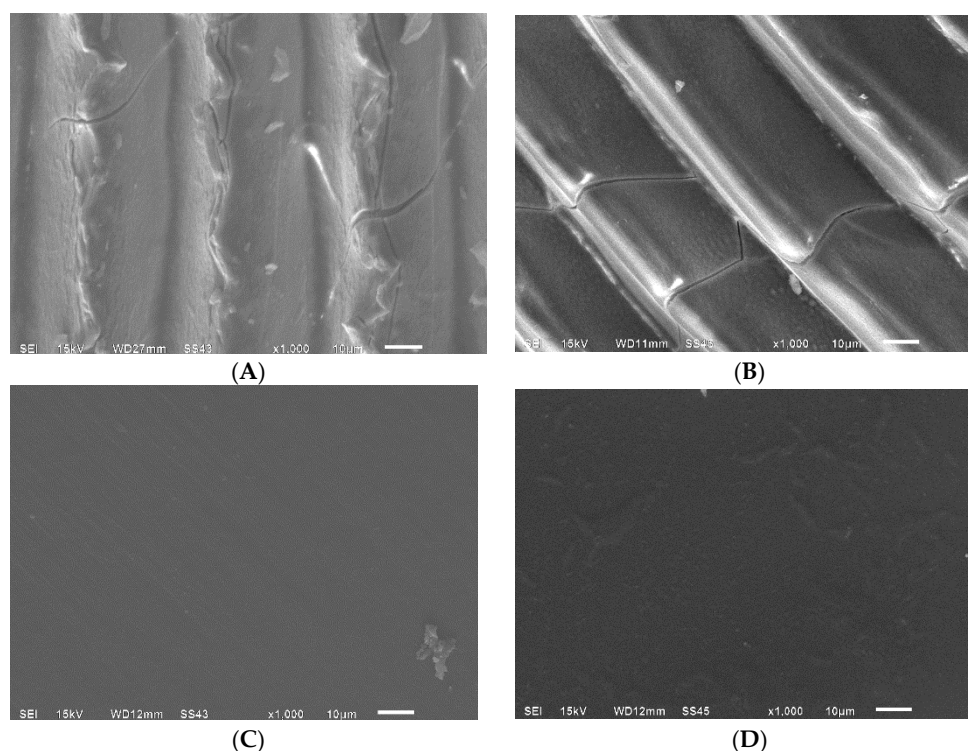


Figure 2. SEM images of the pristine top side of FS before (A) and after (B) dye absorption, and of the bottom side of FS before (C) and after (D) dye absorption.

EDS identified the presence of C, O, N, Na, Mg, P, S and Ca, and quantified their amounts in weight percentages as in Table 4, which reinforces the differences in chemical composition in different places due to the heterogeneous structure of the FS. The higher contents of C, O and N at the bottom side of the FS indicate the presence of high protein organics called collagen; in contrast, the top side having high concentration of Ca, S, P with significant Ca:P ratio (1.27 and 2.14 before and after dye absorption, respectively) ensures the presence of inorganic minerals, such as hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, and/or carbonated apatite, $\text{Ca}_{10}(\text{PO}_4)_6\text{CO}_3$ [10]. The positive change in the Ca:P ratio (from 1.27 to 2.14) and negative change in the C and N in the colored FS might be due to leaching organic matters in the presence of acid and salt coupled with temperature application during the absorption process.

Table 4. EDS elemental analysis (expressed in percentage of weight) and comparisons with other reported findings.

		Elements	C	N	O	Na	Mg	P	S	Ca	References
Criteria	P	T	27.02	16.65	38.62	0.7	0.53	7.25	NA	9.22	This study
		B*	55.41	23.49	21.10	NA	NA	NA	NA	NA	
	C*	T	4.64	3.34	53.52	NA	NA	12.0	0.79	25.71	
		B	48.58	18.72	32.72	NA	NA	NA	NA	NA	
Rohita	NA*	NA	46.08	26.64	24.86	NA	NA	0.91	0.24	1.27	[8]
Tilapia	NA	NA	45.7	NA	38.9	0.2	0.2	5.6	NA	9.4	[10]
E* bass	NA	NA	40.1	0.54	24.0	NA	NA	8.47	NA	9.6	[15]

Notes: P = Pristine FS, C = Colored FS, T = Top side of FS, B = Bottom side of FS, E = European, NA = Not applicable.

3.1.3. Thermo-Gravimetric Analysis of FS

Thermo-gravimetric analysis (TGA) of pristine FS accounted initial weight loss 14.2% between 25 °C and 150 °C due to evaporation of adsorbed water (to 110 °C) and of the hydrogen-bonded (absorbed) water (to 150 °C). By 246 °C, more than 3% weight was lost from the inorganic lattice structure and decomposition of light volatile compounds of FS [21]. The maximum loss of weight (almost 0.2%/°C) was noted at 332.5 °C, and 285 °C was set as the thermal degradation temperature. In the temperature domain of 285 °C to 400 °C, collagen polymeric chains might melt, degrade and decompose according to Torres et al. [27]. Decomposition of other macromolecular substances and evolution of gases take place after 400 °C, and finally, about 50% residues of inorganic apatite was obtained [28,29].

3.1.4. FTIR

The FTIR of pristine and pulverized scales presented the absorptions characteristic to peptide (amide) groups from polymer chain amino-acids, -NH-C(=O)-, labelled as Amide A, Amide I and Amide II, along with the bands typical to absorbances for phosphate and carbonate ions in the apatite lattice containing hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, and calcium carbonate, CaCO_3 . These are presented in Figure 3A [30]. Nonetheless, after grinding FS to 75 μ , the intensity of inorganic component was augmented as the ratio between the major phosphate absorption and that of Amide I increased over three folds (to 7.57) as compared to that of the raw scale (2.38 for as such) (Figure 3B).

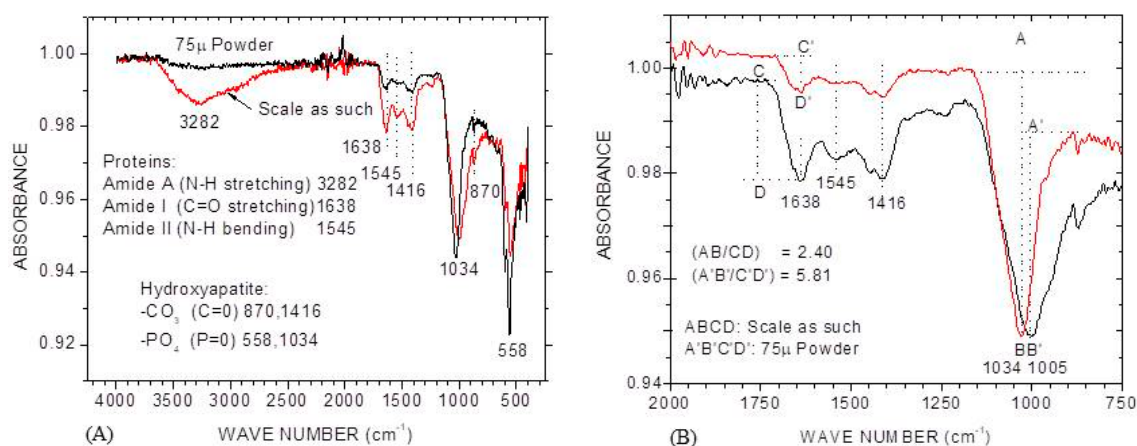


Figure 3. FTIR spectra of the pristine (as such) FS (A), and after grinding to 75 μ powder (B).

An important observation to note is that apatite components were the major players in reacting with sodium salts of sulfonic acid dyes. After dye absorption, although the Amide I and Amide II absorptions were present both in raw and in colored scales, the phosphate and carbonate anions were absent due to their migration in water as sodium soluble salts. Figure 4 illustrates the FT-IR spectra of blue scales colored with acid blue 45 dye. Certain absorptions of the blue acid dye might be identified in the FT-IR spectrum of blue scales (Figure 4A), while this spectrum is lacking $-\text{PO}_4$ and $-\text{CO}_3$ anion bands (Figure 4B).

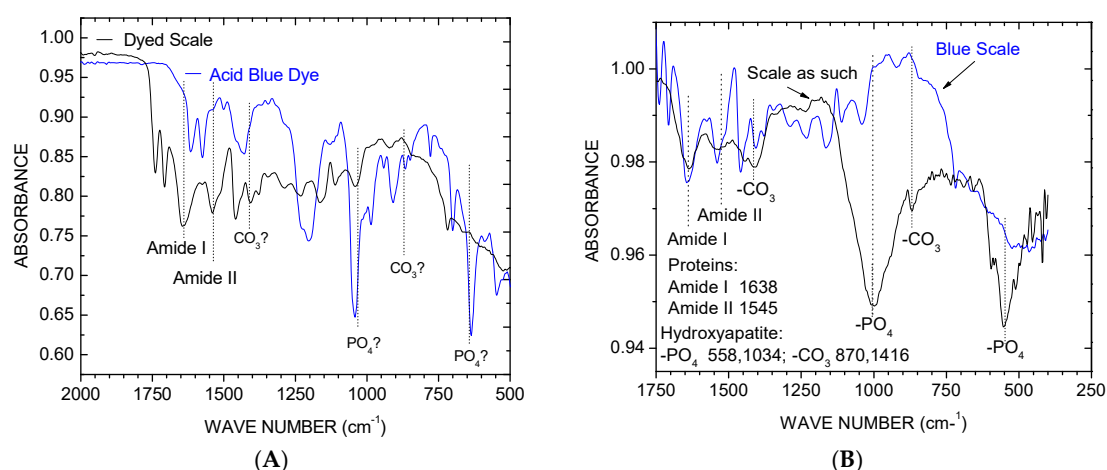


Figure 4. FTIR spectra of the blue colored fish scale and the AB45 dye used for scale coloration (A), and the pristine (as such) FS and of the blue colored scale (B) [30].

3.2. Effects of Process Parameters

3.2.1. Effect of the Initial Dye Concentration and the Amount of FS Used

Several dye stock solutions and amounts of FS (Table 3) were used to study their effects on dye removal efficiency. Figure 5A shows that the dye absorbed in scales was increased for all dyes with the increase of the dye initial concentrations. On the other hand, the dye removal percentage remained almost constant, because the added dye molecules with the increase of dye initial concentration were absorbed by FS in their free dye binding sites. On the other hand, dye loading in scales with the increase of the FS amounts decreased (Figure 5B), and removal percentages increased until saturation. The dye removal percentages for AR1, AB45 and AY127 were around 44%, 64% and 84%, and maximum dye absorption in FS for those dyes were 1.8, 2.7 and 3.4 mg/g, respectively. Marrakchi et al. [8] found a similar trend while examining those effects on reactive orange 16 and methylene blue dye removal by carbonized FS. The concentration difference between dyes and scale amounts determines the mass transportation between sorbents and sorbates and directs the dye molecules to move based on the attainment of equilibrium, dye aggregation and saturation, which, however, might be subjected to other process variables, such as time, temperature, salinity, pH, and surfactant, if used [8,13,14,16,21].

Table 5 provides a close scenario of dye removal capacities by different FS along with other sorbents. It is perceived that the proposed FS showed poor performance in terms of dye absorption, though had satisfactory dye removal percentages for low initial dye concentrations. Several notes are mentioned in this regard:

- (1) The type of dyes and FS used in the present study are different from the literature and thereby, may produce different results, as shown in Table 5.
- (2) To study the effect of amount of FS used, it was considered a high amount of FS (25 to 100 gm/L) compared to other studies (4 g/L at most) mentioned in Table 5. This is because the absorption process is held by electrostatic attraction and amounts of active dye binding sites present in the FS, whereas the adsorption process (all other studies in Table 5) is a physical process. So, the amount of dye binding sites, viz., collagens, are responsible for the dye absorption. In the present attempt, FS were not processed, and TGA data has shown that with 50% inorganics, and 17% moisture and volatile matters, only 33% accounted for collagen, which was later depleted to some extent due to leaching. Therefore, the available dye absorbing collagen was not much, as compared to the whole amount of scales used. Consequently, a high denominator value (X_0) in Equation (2), provided a low q_e value, dye absorption by sorbent (FS). On the contrary, most of the studies cited above processed FS through heat and/or chemical treatments that resulted in moisture and

volatile evaporations; for instance, Ooi et al. [21] noticed that application of high heat, such as 800 °C, ended up with 8.6% ash content, and Marrakchi et al. [8] yielded 12.18 g carbonized FS out of 100 g by heating at 600 °C. Ultimately, these processes came up with high removal capacities, since the density gets lower (the volume remained almost the same but the weight decreased significantly). The variations in dye removal performances among dyes might be due to the bulky chemical structures, chemical functionalities and size of dyes. Though, AY127 has a higher molecular weight (803.63) than AR1 (509.42) and AB45 (474.33), its linear structure enables an easy penetration in FS, compared to penetration of the bulky structures of the other two dyes.

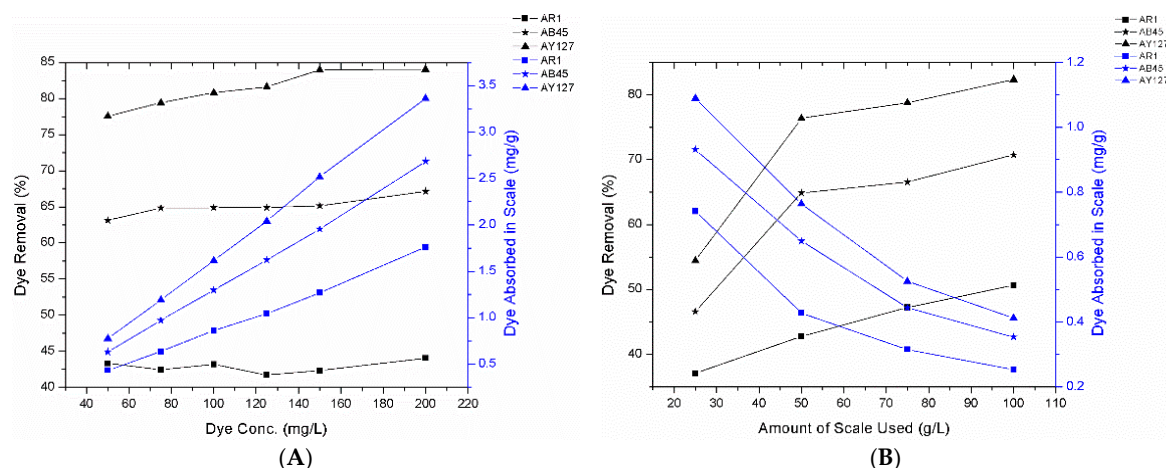


Figure 5. Effect of initial dye concentration (A) (1 h process time, 50 g/L large pulverized FS, and 60 °C) and amounts of scale used (B) (1 h process time, 50 gm/L dye solution, and 60 °C temperature).

Table 5. Comparison of dye binding by FS and other adsorbents.

Sorbent Types		Sorbates	q _e in mg/g	References
Fish Scales	Activated carbon	Reactive blue 5G	149.13	[31]
	Activated carbon	Blue 5G	0.88	[32]
	Palm bark	Methylene blue	2.66	[33]
	Eucalyptus	Methylene blue	2.06	[33]
	<i>Tilapia nilotica</i>	Selenium	1.02	[34]
	<i>Labeo rohita</i>	Brilliant reactive red 2	3.19	[7]
	<i>Labeo rohita</i>	Malachite green	38.46	[16]
	Tilapia fish	Ponceau 4R	116	[6]
	Mixed FS	Acid blue 113	145.3–157.3	[21]
	<i>Pogonias cromis</i>	AR1, AB45, AY127	1.8, 2.7 and 3.4, respectively	This study

3.2.2. Effect of Scale Size and Contact Time on Acid Dye Removal

FS size and the length of contact time had significant effect on the dye removal performance. Performance increased drastically with the increase of FS size (75, 149, 250, 425 μ and larger, as well as pristine whole scales) (Figure 6A), which contradicted the findings for the adsorption process [7,10,16,21]. This disparity in the finding might be explained from the FT-IR spectra, which show the organic content of FS responsible for absorption is reduced while pulverized; however, it outlines future research to reveal the underlying reason behind depletion of organic content. In fact, the absorption does not depend upon the surface area but on the availability of active dye binding sites, which firmly attach the dye molecules by different types of chemical bonding such as covalent bond, and hydrogen bond. On the other hand, adsorption is correlated to the surface area of sorbents and the distribution of pores

on the surface. Pulverization of FS augments specific surface area of FS leading to accumulation of more dye molecules [35]. The contact time (0.25, 0.5, 1, 1.5, 2 and 2.5 h) also had significant impact on dye removal efficiency until saturation (Figure 6B). The cationic functional groups of collagen have strong affinity to the anionic acid dyes; as a result, mass transfer rate was significantly high in the first 30 min and after an hour, it becomes constant, posing the achievement of equilibrium [16]. Therefore, lower equilibrium time and better absorption by whole pristine scales suggest that the present process is time saving and cost effective. Additionally, these colored scales can be used for beautification purpose, such as designing furniture, upholstery and apparel [36].

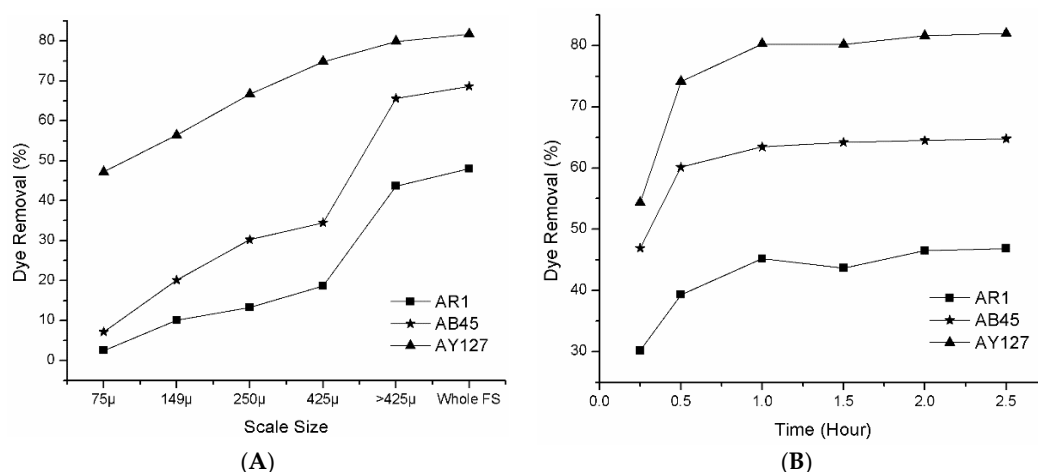


Figure 6. Effect of scale size (A) and of contact time (B) on % dye removal (50 mg/L dye solution, 60 °C temperature).

3.2.3. Effects of Temperature, Salt, Acid, Additives (Acid and Salt), Stirring, and Vacuum on the Anionic Acid Dye Removal

As presented before, Table 2 summarizes the experimental conditions of different process to investigate the effects of temperature, salt, acid, additives (acid and salt), stirring, and vacuum. Figure 7 indicates that the effects of all these variables across all dyes are consistent. The temperature increase positively influenced the absorption performance, indicating an endothermic absorption process [16,37]. The temperature assists the increase of the pore size of the material by swelling, enhances the mobility of dye molecules and reduces the repulsive forces acting on them, enabling the dye molecules to get into the sorbent system easily [16,18]. Although the dye solution for wool dyeing practically contains salt and acid, experiments were conducted without acid and salt to study their contribution in the absorption process. Also, as illustrated in Figure 7, the performance is highly significant in the case of the dye solution void of any additives for all three dyes: AR1 (44 to 64%), AB45 (65 to 89%) and AY127 (79 to 92%).

The actual blue wastewater samples before and after dye absorption by FS, in the case of the dye solution free of any additives, are pictured in Figure 8A,B, respectively. This suggests that the addition of salt and/or acid or their combination might engage some dye binding sites of FS resulting in an inferior performance. The pH of acidic wastewaters (pH = 2.33 for AB45, and pH = 2.5 for AR1 and AY127) in the absence of salt promoted the dye up-take, by making FS more positively charged, which draws the negatively charged sulfonate ($-\text{SO}_3^-$) groups of dyes and vice versa [7,21]. On the other hand, the salt in the absence of the acid negatively affected the process performance implying that probably salt is the one parameter which might involve some dye binding sites. Stirring improved the dye removal percentage considerably, because it helped reduce the diffusional and hydrodynamic boundary layers on FS, which resist the mass transferring process from aqueous solution to the absorbent [38,39]. Finally, for each dye type, the treatment baths were kept in vacuum for 1.5 h after

having completed the usual absorption process to allow the dye molecules to come in intimate contact with the scales. However, the experiments came up with slightly discouraging outcomes.

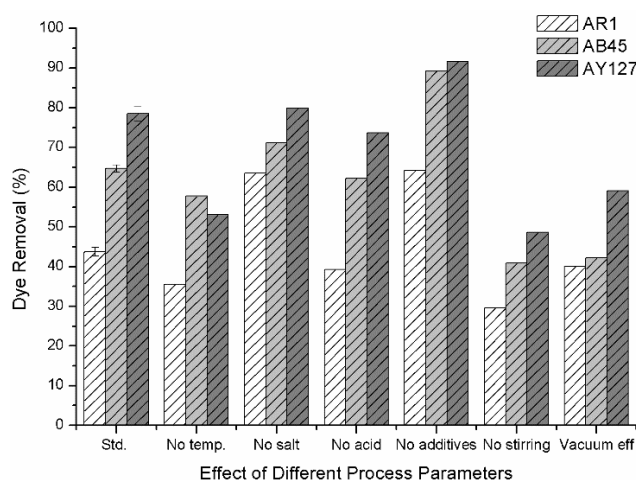


Figure 7. Effect of other process parameters, where std is the control and defined in Table 2.

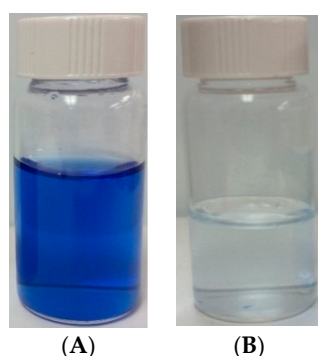


Figure 8. Sample wastewater before (A) and after (B) the absorption process.

3.3. Statistical Analysis

The results showed that all the main effects (dye type and process parameters) and their interactions (dye type X process) were statistically significant (p -value < 0.0001) indicating that all the factors have significant effect on the model. The population least square means (LSM) for each of the interaction combinations were also found to be significantly different from 0. Additionally, the effects of process parameters within each were tested and found to be statically significant. Dunnett's pairwise comparisons between the control and the other processes revealed that: (1) For AR1, vacuum and no acid treatments made no significant difference (p -values 0.0710 and 0.1732) on the % dye removal when compared with standard process; (2) for AB45, there was no significant difference for the process without acid when compared with standard process (p -value 0.4728); and (3) for AY127, there were no significant differences for the processes without salt and acid when compared with standard process (p -values 0.9099 and 0.0506). It also found that the application of temperature and stirring had significant positive effect on the improvement % dye removal, which was also highly significant in case of no additives (acid and salt together) treatment level for all dyes. Although the presence of acid (no salt treatment level) had overall positive effect on the removal performance for all three dyes, the result was not statistically significant for AY127; on the other hand, application of vacuum showed negative effect for all dyes; nonetheless, it was not significant for AR1. The presence of salt (no acid treatment level) had a slight negative effect; however, no statistically significant deviations were found for all dye types when compared to the control group. The results might not be very reliable, because there were not enough replications for the response variable.

4. Conclusions

In the present research, the viability of FS for the removal of acid dyes by absorption process was investigated by physiochemical characterization (SEM-EDS, TGA and FT-IR). The dye removal performance was also assessed, and absorption process was optimized considering different process variables, such as dye initial concentration, amount of FS used, scale size, contact time, temperature, additives (salt and acid), stirring, vacuum.

- SEM-EDS identified the structural and chemical variation in FS. TGA showed that mineral content accounted for 50% of the FS, and the rest was moisture and organic volatile components. FTIR confirmed absorptions characteristic of dyes.
- Dye absorption increased with initial dye concentration, amount of scale used, and decreased due to additives. The best and the worst dye removal performances were found for the dye solution without any additives and fine pulverized scales, respectively.
- The contact time and the temperature allowed the absorption process to achieve equilibrium within 60 min, and it was endothermic.
- The statistical analysis showed strong evidence that the process parameters, dyes, and dye type \times process parameters interactions had significant effect on the dye absorption. Although the dye loading on scales is not significantly high, the dye removal percentage is appreciable.

Additionally, no significant preparation of scales, shorter equilibrium time, ability to use whole scales and no chemical usage suggest that the present process is easy and cost saving. The colored scales can later be used for ornamentation purposes [36]. Future research can be performed to optimize the performance such as correlating absorption and adsorption process by analyzing surface characteristics (i.e., specific area, porosity, pore diameter and total pore volume).

Author Contributions: S.M.F.K. and I.I.N. developed the idea and designed the research. S.M.F.K. undertook the experiments, prepared and drafted the manuscript. R.C. and S.B. interpreted the graph. B.D.M. used and designed statistical analysis. L.D.R. and J.T.K. revised and edited the final draft. All authors read and approved the final manuscript.

Acknowledgments: Thanks to Louisiana State University (Baton Rouge, LA, USA) for proving research support. The authors are also thankful to Big D, Franklin, LA, USA for donating fish scales, Evelyn (Gutierrez) Watts and Cindy Cordoba Arroyo for supporting in the collection of fish scales, and Qunglin Wu for laboratory support in preparing fish scales.

Conflicts of Interest: The authors declare no conflicts of interests.

References

1. Hinrichsen, D.; Tacio, H. The coming freshwater crisis is already here. In *The Linkages between Population and Water*; Woodrow Wilson International Center for Scholars: Washington, DC, USA, 2002; pp. 1–26.
2. Saxena, S.; Raja, A.; Arputharaj, A. Challenges in Sustainable Wet Processing of Textiles. In *Textiles and Clothing Sustainability*; Springer: Berlin, Germany, 2017; pp. 43–79.
3. Kabir, S.; Chakraborty, S.; Hoque, S.; Mathur, K. Sustainability Assessment of Cotton-Based Textile Wet Processing. *Clean Technol.* **2019**, *1*, 232–246. [[CrossRef](#)]
4. Grady, C.L.J.; Daigger, G.T.; Love, N.G.; Filipe, C.D. *Biological Wastewater Treatment*; CRC Press: Boca Raton, FL, USA, 2011.
5. Gupta, V.; Carrott, P.; Ribeiro Carrott, M.M.L.; Suhas. Low-cost adsorbents: Growing approach to wastewater treatment—A review. *Crit. Rev. Environ. Sci. Technol.* **2009**, *39*, 783–842. [[CrossRef](#)]
6. Zhu, K.; Gong, X.; He, D.; Li, B.; Ji, D.; Li, P.; Peng, Z.; Luo, Y. Adsorption of Ponceau 4R from aqueous solutions using alkali boiled Tilapia fish scales. *RSC Adv.* **2013**, *3*, 25221–25230. [[CrossRef](#)]
7. Begum, H.A.; Kabir, M.H. Removal of Brilliant Red from Aqueous Solutions by Adsorption on Fish Scales. *Dhaka Univ. J. Sci.* **2013**, *61*, 7–12. [[CrossRef](#)]
8. Marrakchi, F.; Ahmed, M.J.; Khanday, W.; Asif, M.; Hameed, B. Mesoporous carbonaceous material from fish scales as low-cost adsorbent for reactive orange 16 adsorption. *J. Taiwan Inst. Chem. Eng.* **2017**, *71*, 47–54. [[CrossRef](#)]

9. Neves, C.; Scheufele, F.; Nardino, A.; Vieira, M.; da Silva, M.; Módenes, A.; Borba, C. Phenomenological modeling of reactive dye adsorption onto fish scales surface in the presence of electrolyte and surfactant mixtures. *Environ. Technol.* **2018**, *39*, 2467–2483. [[CrossRef](#)] [[PubMed](#)]
10. Ribeiro, C.; Scheufele, F.B.; Espinoza-Quinones, F.R.; Módenes, A.N.; da Silva, M.G.C.; Vieira, M.G.A.; Borba, C.E. Characterization of *Oreochromis niloticus* fish scales and assessment of their potential on the adsorption of reactive blue 5G dye. *Colloids Surf. A Physicochem. Eng. Asp.* **2015**, *482*, 693–701. [[CrossRef](#)]
11. Zynudheen, A. Utilization of Fishery Waste in India. Central Institute of Fisheries Technology: Kerala, India, 2010.
12. Vieira, E.F.; Cestari, A.R.; Carvalho, W.A.; Oliveira, C.d.S.; Chagas, R.A. The use of freshwater fish scale of the species *Leporinus elongatus* as adsorbent for anionic dyes. *J. Therm. Anal. Calorim.* **2012**, *109*, 1407–1412. [[CrossRef](#)]
13. Marrakchi, F.; Auta, M.; Khanday, W.; Hameed, B. High-surface-area and nitrogen-rich mesoporous carbon material from fishery waste for effective adsorption of methylene blue. *Powder Technol.* **2017**, *321*, 428–434. [[CrossRef](#)]
14. Chakraborty, J.; Dey, S.; Halder, U.C. An eco-friendly bio-sorbent derived from fish (Carp) scale: A study of commercial dye removal. *Int. J. Sci. Eng. Res.* **2016**, *7*, 5.
15. Uzunoğlu, D.; Özer, A. Adsorption of Acid Blue 121 dye on fish (*Dicentrarchus labrax*) scales, the extracted from fish scales and commercial hydroxyapatite: Equilibrium, kinetic, thermodynamic, and characterization studies. *Desalin. Water Treat.* **2016**, *57*, 14109–14131. [[CrossRef](#)]
16. Chowdhury, S.; Das Saha, P.; Ghosh, U. Fish (*Labeo rohita*) scales as potential low-cost biosorbent for removal of malachite green from aqueous solutions. *Bioremediation J.* **2012**, *16*, 235–242. [[CrossRef](#)]
17. Eaglen, M.; Pollak, J. *How to Save Money, Reform Processes, and Increase Efficiency in the Defense Department*; Heritage Foundation: Washington, DC, USA, 2011.
18. Mahapatra, N.N. *Textile Dyes*; Woodhead Publishing: New Delhi, India, 2016.
19. Ogawa, M.; Portier, R.J.; Moody, M.W.; Bell, J.; Schexnayder, M.A.; Losso, J.N. Biochemical properties of bone and scale collagens isolated from the subtropical fish black drum (*Pogonia cromis*) and sheepshead seabream (*Archosargus probatocephalus*). *Food Chem.* **2004**, *88*, 495–501. [[CrossRef](#)]
20. Negulescu, I.I. *Laboratory Procedure*; Louisiana State University: Baton Rouge, LA, USA, 2017.
21. Ooi, J.; Lee, L.Y.; Hiew, B.Y.Z.; Thangalazhy-Gopakumar, S.; Lim, S.S.; Gan, S. Assessment of fish scales waste as a low cost and eco-friendly adsorbent for removal of an azo dye: Equilibrium, kinetic and thermodynamic studies. *Bioresour. Technol.* **2017**, *245*, 656–664. [[CrossRef](#)]
22. Bhuiyan, M.R.; Ali, A.; Islam, A.; Hannan, M.; Kabir, S.F.; Islam, M. Coloration of polyester fiber with natural dye henna (*Lawsonia inermis* L.) without using mordant: A new approach towards a cleaner production. *Fash. Text.* **2018**, *5*, 2. [[CrossRef](#)]
23. Kabir, S.F. Waste Management by Waste: Removal of Acid Dyes from Wastewaters of Textile Coloration Using Fish Scales. Master's Thesis, Louisiana State University, Baton Rouge, LA, USA, 2018. in press.
24. Calloway, D. Beer-lambert law. *J. Chem. Educ.* **1997**, *74*, 744. [[CrossRef](#)]
25. Rodger, A. Concentration Determination Using Beer-Lambert Law. In *Encyclopedia of Biophysics*; Springer: Berlin, Germany, 2013; pp. 360–361.
26. Lagler, K.F. Lepidological studies 1. Scale characters of the families of Great Lakes fishes. *Trans. Am. Microsc. Soc.* **1947**, *66*, 149–171. [[CrossRef](#)]
27. Torres, F.G.; Troncoso, O.P.; Amaya, E. The effect of water on the thermal transitions of fish scales from *Arapaima Gigas*. *Mater. Sci. Eng. C* **2012**, *32*, 2212–2214. [[CrossRef](#)]
28. Mota, J.A.; Silva, E.S.; Vieira, E.F.; Sussuchi, E.M.; Cestari, A.R. A highly efficient adsorbent synthesized by reactive depositions of chitosan layers on fish scale collagen—Hydrodynamic swelling and dichlorophenol derivative sorption evaluated by continuous long-term solution microcalorimetry. *J. Environ. Chem. Eng.* **2013**, *1*, 480–485. [[CrossRef](#)]
29. Chakraborty, R.; Bepari, S.; Banerjee, A. Application of calcined waste fish (*Labeo rohita*) scale as low-cost heterogeneous catalyst for biodiesel synthesis. *Bioresour. Technol.* **2011**, *102*, 3610–3618. [[CrossRef](#)]
30. Kabir, S.; Rashid, T.U.; Negulescu, I.I. Gelation of Textile Dye Solution Treated with Fish Scales. *Gels* **2019**, *5*, 37. [[CrossRef](#)] [[PubMed](#)]
31. Martins, T.D.; Schimmel, D.; dos Santos, J.o.B.; da Silva, E.A. Reactive blue 5G adsorption onto activated carbon: Kinetics and equilibrium. *J. Chem. Eng. Data* **2012**, *58*, 106–114. [[CrossRef](#)]

32. Al-Degs, Y.; Khraisheh, M.; Allen, S.; Ahmad, M. Adsorption characteristics of reactive dyes in columns of activated carbon. *J. Hazard. Mater.* **2009**, *165*, 944–949. [[CrossRef](#)] [[PubMed](#)]
33. Sun, L.; Wan, S.; Luo, W. Biochars prepared from anaerobic digestion residue, palm bark, and eucalyptus for adsorption of cationic methylene blue dye: Characterization, equilibrium, and kinetic studies. *Bioresour. Technol.* **2013**, *140*, 406–413. [[CrossRef](#)]
34. Kongsri, S.; Janpradit, K.; Buapa, K.; Techawongstien, S.; Chanthai, S. Nanocrystalline hydroxyapatite from fish scale waste: Preparation, characterization and application for selenium adsorption in aqueous solution. *Chem. Eng. J.* **2013**, *215*, 522–532. [[CrossRef](#)]
35. Iqbal, J.; Wattoo, F.H.; Wattoo, M.H.S.; Malik, R.; Tirmizi, S.A.; Imran, M.; Ghangro, A.B. Adsorption of acid yellow dye on flakes of chitosan prepared from fishery wastes. *Arab. J. Chem.* **2011**, *4*, 389–395. [[CrossRef](#)]
36. Stannard, C.R.; Kabir, S.M.F. Fishy finery. In Proceedings of the 2018 ITAA Annual Conference Proceedings: Design Presentation, Cleveland, OH, USA, 5–9 November 2018.
37. Al-Degs, Y.S.; El-Barghouthi, M.I.; El-Sheikh, A.H.; Walker, G.M. Effect of solution pH, ionic strength, and temperature on adsorption behavior of reactive dyes on activated carbon. *Dyes Pigment.* **2008**, *77*, 16–23. [[CrossRef](#)]
38. Adeyemo, A.A.; Adeoye, I.O.; Bello, O.S. Adsorption of dyes using different types of clay: A review. *Appl. Water Sci.* **2017**, *7*, 543–568. [[CrossRef](#)]
39. Moreira, R.; Peruch, M.; Kuhnen, N. Adsorption of textile dyes on alumina. Equilibrium studies and contact time effects. *Braz. J. Chem. Eng.* **1998**, *15*. [[CrossRef](#)]



© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).