Waste Management by Waste: Removal of Acid Dyes from Wastewaters of Textile Coloration Using Fish Scales

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WASTE MANAGEMENT BY WASTE: REMOVAL OF ACID DYES FROM WASTEWATERS OF TEXTILE COLORATION USING FISH SCALES

A Thesis

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Master of Science in

The Department of Textiles, Apparel Design and Merchandising

by

S M Fijul Kabir
B.S., University of Chittagong, Bangladesh, 2013
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I am grateful to my parents, brothers, sisters, teachers, friends and relatives for their prayers, motivations, best wishes and supports. I always find them beside me in any of my crisis and hardships that provides me the life-giving force. Finally and absolutely, all praise is due to the Almighty, Who has blessed me with His mercy.
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ABSTRACT

Removal of hazardous acid dyes by economical process using low-cost bio-sorbents from wool industry wastewaters is of a pressing need, since it causes skin and respiratory diseases and disrupts other environmental components. Fish scales (FS), a by-product of fish industry, a type of solid waste, are usually discarded carelessly resulting in pungent odor and environmental burden. In this research, the FS of black drum (*Pogonias cromis*) were used for the removal of remnant acid dyes (acid red 1, acid blue 45 and acid yellow 127) from wool industry wastewaters by absorption process with a view to valorizing fish waste and mutual management of both fish by-product and wastewaters of wool industry. The dye absorption process was investigated considering process variables such as dye concentration, amount of fish scale (FS) used, contact time, FS size, temperature, acid (pH), salt, combination of salt and acid, stirring and vacuum as well as the physicochemical characterization of absorbent was conducted through scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), thermogravimetric analysis (TGA), fourier-transform infrared spectroscopy (FT-IR) analyses. Microscopic characterization revealed the beautiful architecture of FS with different chemical compositions, TGA revealed that about 50% of scale was mineral and 50% polymer, and finally the evidence of absorption bands of the functional groups for dyes in the colored FS was confirmed by FT-IR analysis. Optimization of process variables represented that maximum dye removal percentages for AR1, AB45, and AY127 were 63.5, 89.3 and 92, and maximum absorptions were 1.8, 2.7 and 3.4mg/g, respectively. The results followed almost the same trend across all three dyes for all process variables. Dyes without any additives (salt and acid) exhibited the best removal performance in terms of percentage of removal, whereas the most unusual and inferior outcomes were found in case of fine powdered FS. Two-way analysis of variance (ANOVA) for different dyes and process parameters showed
dye types, process parameters and their interactions (dye type X process) had significant effect to the ANOVA model. Over the time, after absorption process the remaining wastewaters formed gelatin probably due to leaching some collagen from the scales. Additional study for characterization of the gel was performed through rheological analysis to find melting point of the gel.
CHAPTER 1: INTRODUCTION

1.1 Scarcity of Freshwater

Over two thirds of our planet is covered by water; however, only three percent is freshwater. Pathetically, 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 (Hinrichsen & Tacio, 2002). Figure 1.1 nicely illustrates world’s freshwater reserve scenario.

Figure 1.1: The world’s freshwater resources (Conradin, 2012, p. 1)

Worldometers (2017) reports that in 2017 freshwater consumption was over $9 \times 10^9$ millions of liters, and more than half of that amount was used for industrial purposes that cause a crisis of additional 64000 billion liters of water each year. According to the recent report of World Health Organization, approximately one third of all healthcare facilities of low- and middle-income countries suffer from lack of safe water (Bartram et al., 2015). The World Economic Forum has indicated the scarcity of water as the number one risk based on its impact on society. The impact is omnipresent, and countries from underdeveloped to developed in terms of economy are seriously facing the scarcity of water (Cann, January 14, 2016). Moreover, unequal distributions
and consumptions of freshwater among different countries make the water stressed countries more vulnerable. For instance, Emirates Industrial Bank states that the United Arab Emirates (UAE) accounts for the highest per capita consumption of water in the world, although UAE itself is a water stressed country (Chaudhury, July 22, 2005). The countries using the most water include China, USA, Brazil, Russia, Mexico along with many others and statistics indicate that massive urbanization, industrialization, and population growth have positive correlation with water consumption. On the other hand, other parts of the world are at the brink of severe water crisis (Contractor, 2016). Though the rain water is sufficient to meet world’s water requirements, we hardly can utilize for want of proper methods or technology to reserve them. Therefore, groundwater is the main source of freshwater, which is considered as a nonrenewable natural resource. It has been reported that by 2025 more than half and by the middle of this century more than two thirds of the countries around the world might encounter water crisis due to depletion of groundwater levels (Shakhawat Chowdhury & Husain, 2006; Rogers, 2008).

1.2 Overview of Water Consumption

It is already evident that industries consume more than two thirds of the freshwater withdrawal. These industries include chemical, agriculture, leather, cosmetic, steel, refinery, cement as well as textile processing. Figure 1.2 shows water consumption by different industries. They not only consume significant amount of water for processing, but also pollute water relentlessly. Among them, textile processing industries having the greatest supply chain ranging from fiber to apparel production involve a lot of processes, which require water at various wet treatment stages. In this regard, textile industry is one of the water-thirstiest and water polluting industries (Saxena, Raja, & Arputharaj, 2017). Besides processing of textiles like desizing, scouring, bleaching, dyeing and washing, water is also used for boiler steam generation. It is
estimated that for cotton processing it requires about 80 to 150 liters water for processing 1 kg of fabric.

Figure 1.2: Water consumption by different industry (Shailesh, 2011, p. 1)

Again, the consumption of the water is influenced by water properties, machine designs, machine types, process types, and the materials to be processed. During processing textiles, varieties of chemicals including dyes, pigments, acids, bases, sequestering agents, salts, surfactants, softeners, fixing agents, formaldehydes are used that deteriorate water qualities. All the processes are not equally responsible for wastewater generation; for instance, boiler water is not polluted; again, water of peroxide bleaching is less injurious compared to the water used for sizing, desizing, and coloration. The consumption of water of different processes are different such as pretreatment (38%), dyeing, printing and finishing (38%), and others (24%) (Bhuiyan, Rahman, Shaid, Bashar, & Khan, 2016; Shaikh, 2009).

1.3 Nature of Textile Wastewaters and their Harmful Effects

The high water consumption for textile processing is itself a big concern for the scientific community for what a significant portion of biotic components of aquatic biome are facing the reality of paucity of freshwater, what if the situation is buttressed to more deterioration
by discharging the textile wastewaters containing noxious chemicals, which have both tangible
and intangible impacts on all the basic abiotic components of environment viz. hydrosphere,
lithosphere and atmosphere (Grady Jr, Daigger, Love, & Filipe, 2011). Undoubtedly, these
sufferings would not have any bound. Generation of textile wastewaters is seriously condemned,
because it severely affects water qualities. Patel and Vashi (2015) report that twenty two quality
parameters are used to assess water qualities. They also enlist the effluents engendered in various
textile processes as mentioned in Table 1.1.

<p>| Table 1.1: Effluents generated in different textile processes (Patel &amp; Vashi, 2015, p. 10) |</p>
<table>
<thead>
<tr>
<th>Processes</th>
<th>Reagents/Chemicals used</th>
<th>Effluents generated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sizing</td>
<td>Starch, waxes, carboxymethyl cellulose (CMC), polyvinyl alcohol (PVA), wetting agents</td>
<td>High in Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD)</td>
</tr>
<tr>
<td>Desizing</td>
<td>Starch, CMC, PVA, fats, waxes, Pectin</td>
<td>High in BOD, COD, suspended solid (SS), Dissolved Solid (DS)</td>
</tr>
<tr>
<td>Bleaching</td>
<td>Sodium hypochlorite, Cl₂, NaOH, H₂O₂, acids, surfactant, Na₂SiO₃, sodium phosphate,</td>
<td>High alkalinity, high SS</td>
</tr>
<tr>
<td></td>
<td>cotton fiber</td>
<td></td>
</tr>
<tr>
<td>Mercerizing</td>
<td>Sodium hydroxide, cotton wax</td>
<td>High pH, low BOD, high DS</td>
</tr>
<tr>
<td>Dyeing</td>
<td>Dyestuffs urea, reducing agents, oxidizing agents, acetic acid, detergents, wetting</td>
<td>Strong colored, high BOD, high DS, low SS, low heavy metals, high salinity, electric conductivity</td>
</tr>
<tr>
<td></td>
<td>agents</td>
<td></td>
</tr>
<tr>
<td>Printing</td>
<td>Pastes, urea, starches, gums, oils, binders, acids, thickeners, cross-linkers, reducing</td>
<td>Highly colored, high BOD, oily appearance, high suspended solid, slightly alkaline, low BOD</td>
</tr>
<tr>
<td></td>
<td>agents, alkali</td>
<td></td>
</tr>
</tbody>
</table>
The levels of these parameters in textile wastewaters are remarkably high (Hussain, Hussain, & Arif, 2015; Islam et al., 2015). For instance, characterizing textile wastewaters of an apparel industry of Bangladesh, Roy et al. (2010) reported as quoted below.

Total suspended solids (TSS) and total dissolved solid (TDS) was found 100 to 336 mg/L and 1856 to 4356 mg/L, respectively. The recorded pH was 9.6 to 11.2 and temperature 40.5 to 43 OC. Dissolved oxygen (DO), biological oxygen demand (BOD) and chemical oxygen demand (COD) was recorded 0.11 to 0.5, 151.24 to 299.1 and 652.8 to 2304 mg/L, respectively. Electrical conductivity (EC) was monitored 2210 to 6020 µs/cm; salinity was found 1.1 to 3.3 ‰, and color 1890.75 to 5625 PCU. Zinc (Zn), cadmium (Cd), copper (Cu) were 0.0838 to 0.596, 0.0 to 0.0006, 0.018 to 0.1727 ppm, respectively. Except heavy metals concentrations all of the physicochemical parameters were found beyond the standard limit set by department of Environment (DoE). Textile industrial effluents showed the toxic effects on aquatic macrophytes and algae, as aquatic macrophytes could hardly survive 2 days on textile effluents and noticeable reduction of algal concentration was found when grown on textile effluents.

Therefore, if the wastewaters are discharged to the environment without treatment, it becomes life threat for all components of environment. Khan and Malik (2014) documented the pernicious effects due to textile wastewaters. Depletion of dissolved oxygen in water for hydrosulfites and blocking the passage of sunlight due to thick concentration of colors jeopardize the lives under water. Using salt as electrolytes during dyeing and printing raises salinity that causes the soil impotent and futile. Textile industries mostly use low cost synthetic dyes, which are not biodegradable. Some synthetic dyes contain heavy metals. Moreover, dyes containing chlorine are carcinogens, which are responsible for allergic problem. Due to chemical evaporation, when these carcinogens, heavy metals, and other chemicals are exposed to the air, they are absorbed by the body, which, in long run, bring about physiological and biochemical malfunctioning of body’s organs.

1.4 Textile Wastewater Treatments

Along with the growing worldwide environmental concern and human safety, these wastewaters need treatment before being discharged to the environment (Profile, 2010). For
example, Bangladesh Government has imposed strict regulations on using fully capacitated Effluent Treatment Plant (ETP) for textile processing industries. Moreover, the discharged water qualities are continuously monitored (Clemett, 2004). The basic principle of wastewater treatment include separation of suspended and dissolved solids, oxidation of organic and oxygen demanding materials, neutralization, removal of toxic ingredients (Patel & Vashi, 2015). To treat textile wastewaters, different methods such as physicochemical processes (sorption, filtration, ultrafiltration and dialysis, reverse osmosis, ion exchange, evaporation, solvent extraction, oxidation - using chlorine, ozone, hydrogen peroxide, or chlorine dioxide), biological processes (coagulation, foam flotation and photochemical reactions, activated sludge, aerobic and anaerobic treatment, microbial reduction, bacterial treatment), nuclear treatments (irradiation), and acoustical, electrical, and electromagnetic processes (electrodialysis, electrolysis, ultrasonic treatment, and magnetic separation) are available (Gupta, Carrott, Ribeiro Carrott, & Suhas, 2009; Rafatullah, Sulaiman, Hashim, & Ahmad, 2010). Among them the sorption process is now considered as the most effective and economical way to treat textile wastewaters (Uzunoğlu & Özer, 2016).
2.1 The Sorption Process

The mechanism of the sorption process is explained by the phenomenon of donating and accepting electrons between functional groups of sorbents and sorbates; the nature of their interactions decides whether the process is physisorption or chemisorption. In this regard, the sorption process can be described by two phenomena: (1) physical sorption, physiosorption or adsorption and (2) chemical sorption, chemisorption or absorption. The term ‘adsorption’ was firstly introduced by Kayser in 1881 to explain surface accumulation of substances. It is generally referred as the process of concentrating a material onto the surface of another solid material. In physical adsorption, the attraction between the solid phase and adsorbed particles has a physical nature. There is no chemical reaction between the solute and the adsorbent, but they are poorly attached by weak Van der Waal’s forces. In contrast, chemisorption takes place by the electrostatic attraction between solute and sorbate followed by chemical bonding in them. Thus, the chemisorption is an usually irreversible process and occurs in monolayer, whereas the physisorption is reversible, and multilayer is formed here (Gupta et al., 2009). In absorption, the kinetics during absorption and chemical reaction follow same order of reaction. But, the physisorption is an exothermic process, and liberated energy is not equal to the energy of condensation. Moreover, the adsorption process is improved with the increment of surface area and pore size of the adsorbent surface so that molecules of dyes can be anchored and adsorbed by the adsorbents. Since chemisorption process is developed by chemical bonding, it often requires activators to enhance rapid attainment of equilibrium like temperature, agitation, electrolytes. In physisorption, equilibrium reaches considerably fast (Patel & Vashi, 2015).
2.2 Sorbents

McKey (1995) mentions principle features of selecting the sorbents in his book titled ‘Use of Adsorbents for the Removal of Pollutants from Wastewater’: high selectivity, high sorption capacity, longer life, lower in cost, and abundantly available. The selectivity is evaluated based on some measurements; for instance, equilibrium studies, kinetic and diffusional properties, molecular sieve separation, pore size, surface area, and intramicrocrystalline structure. There are abundant research articles of using adsorbents for treating wastewaters. Apart from the research articles, there are good numbers of review articles on different aspects of adsorption; for instance, based on the types of pollutants, the types of adsorbents (Babel & Kurniawan, 2003; Bhatnagar & Minocha, 2006; Bhatnagar & Sillanpää, 2010; Bilal et al., 2013; Crini, 2006; Gupta et al., 2009; Johnson, Jain, Joshi, & Prasad, 2008; Pollard, Fowler, Sollars, & Perry, 1992; Rafatullah et al., 2010; Sharma, Kaur, Sharma, & Sahore, 2011; Vakili et al., 2014; Zolgharnein, Shahmoradi, & Ghasemi, 2011). The contaminants of wastewaters generally include dyes, heavy metals, pesticides, phenolic compounds, polychlorinated biphenyl, radioactive substances, detergents etc. Again, there are different classes of sorbents like synthetic, regenerated, industrial waste, agricultural waste, bio based, inorganic materials etc. However, researchers are constantly looking for new, alternative, low cost bio adsorbents. At this juncture, among the materials used from these textile wastewater treatments, fish wastes are mentioned in the the recent investigations as very promising, environmentally friendly and economically very affordable solutions.

2.3 Overview of Fish Wastes

United Nations reports that global fish consumption reached to its highest level in the last year throughout history, 20kg per capita, and its consumption is increasing gradually (Kinver, 7 July 2016). During processing of these fishes, there are generated huge amounts of fish scales
(FS), which are often discarded and simply considered solid waste. Sharp and Mariojouls (2012) state the global waste generation from fisheries items account 130 million tons per year. Arvanitoyannis and Kassaveti (2008) and (Zynudheen, 2010) enlist the types of fish wastes and their potential uses. The types of fish wastes are mainly heads, bones, skin, scales, fins, viscera and sometimes whole fish, parsley. Their potential fields of applications are animal feeds, biogas, biomass, natural pigments, food industries, cosmetics, ornaments, pharmaceuticals, surgical, effluents treatment, biomedical, chemical, agriculture, photography etc. Arvanitoyannis and Kassaveti (2008) state that roughly 20-40kg scales and more than 50kg skin can be obtained from 1000kg fishes during fillet processing. Nonetheless, the uses are very limited let alone commercial use. Zynudheen (2010) shows uses of negligible amount of scales, which are confined within cosmetic and ornamentation purpose. There are considerable attempts of extracting collagen, chitin or chitosan out of FS, skins and bones, but direct uses of these fish wastes still need to be explored (Alcalde & Fonseca, 2017; Iqbal et al., 2011; Muslim, Rahman, Begum, & Rahman, 2013; Nagai & Suzuki, 2000). The use of FS as absorbents is quite new that has been pioneered by the research of heavy metals adsorption (Basu et al., 2006; Basu, Rahaman, Mustafiz, & Islam, 2007; Moura, Vieira, & Cestari, 2012; Mustafiz, 2003; Nadeem, Ansari, & Khalid, 2008; Srividya & Mohanty, 2009; Villanueva-Espinosa, Hernandez-Esparza, & Ruiz-Trevino, 2001). Afterwards, treatment of textile wastewater by FS has been incepted by extracting chitin and chitosan out of scales through different physiochemical processes (Iqbal et al., 2011).

2.4 Fish Scales for Dye Removal

Using FS for the dye removal is a very recent innovation. The handful of available research works have investigated different attributes like measuring dye removal capacity and its optimization including thermodynamic, kinetic, calorimetric studies of the adsorption process as
well as characterization of scales before and after the adsorption. The studies reviewed regarding these concerns are summarized in Table 2.1. It is found that use of scales of the proposed fish (black drum) in this project, one of the most available fishes in this territory, have not been explored yet for the dye removal process. Moreover, detailed study on the characterization of drum’s scales is not well established to understand chemical behavior of drum’s scales especially during sorption process. For all the above reported works, FS have been treated with different chemicals like NaOH (Vieira, Cestari, Carvalho, Oliveira, & Chagas, 2012; Zhu et al., 2013), sodium hypochlorite (Ribeiro et al., 2015), or EDTA (J. Chakraborty, Dey, & Halder, 2016) or with high temperature (Begum & Kabir, 2013; Ribeiro et al., 2015; Uzunoğlu & Özer, 2016; Vieira et al., 2012; Zhu et al., 2013) with a view to boosting up adsorption capacity. In addition, to obtain maximum specific area of scales, powdered scales of different sizes have been used (Begum & Kabir, 2013; Shamik Chowdhury, Das Saha, & Ghosh, 2012; Ribeiro et al., 2015; Zhu et al., 2013). On the other hand, adopting any physical and/or chemical process involves some investments in terms of money, time, labor. Any forms of investments apparently increase process cost (Eaglen & Pollak, 2011). Through our research, we assessed the performance of drum’s scales as commercial bio absorbent for the purpose of using large volume by skipping physical (heat treatment) or chemical processes (treating with NaOH, NaOCl₂, or EDTA, HCl).
<table>
<thead>
<tr>
<th>Type of FS used</th>
<th>Type of dye used</th>
<th>How the scales were prepared</th>
<th>What were investigated</th>
<th>Maximum dye adsorption capacity and references</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Labeo rohita</strong></td>
<td>Brilliant Red (C. I. Reactive Red 2)</td>
<td>Washed with distilled water, oven dried at 60°C, ground and sieved of different sizes ranging from 0.144 mm to 0.355 mm.</td>
<td>Contact time: 3h, Dye concentration in solutions: 1g/L, Temperature: 30°C, pH: 7.2 (optimum)</td>
<td>Langmuir and Freundlich isotherm model: Fitted, 3.19 mg/gram (Begum &amp; Kabir, 2013)</td>
</tr>
<tr>
<td><strong>Labeo rohita</strong></td>
<td>Malachite Green</td>
<td>Washed with distilled water, sun dried, oven dried at 70°C into crispy, ground into a constant size (100–125 μm).</td>
<td>BET&lt;sup&gt;a&lt;/sup&gt;, SEM&lt;sup&gt;b&lt;/sup&gt;, Dye solution: 1g/L, Contact time: 2h, Temperature: 40°C, pH: 8, Bio adsorbent dose: 2g/L (150rpm agitation)</td>
<td>Langmuir and Freundlich isotherm models: Fitted, 38.46 mg/g (Shamik Chowdhury et al., 2012)</td>
</tr>
<tr>
<td><strong>Labeo rohita</strong></td>
<td>Reactive orange 16</td>
<td>Carbonized FS by electrical furnace under 99.99% N&lt;sub&gt;2&lt;/sub&gt; at 100 cm³/min flow rate and 10°C/min heating rate and then washed with 1 M HCl.</td>
<td>BET, TGA&lt;sup&gt;c&lt;/sup&gt;, X-ray (EDX&lt;sup&gt;d&lt;/sup&gt;), FT-IR&lt;sup&gt;e&lt;/sup&gt;, Dye concentration: (25–400 mg/L), Temperature: (30–50°C)</td>
<td>Freundlich isotherm model: Fitted, 114.2 mg/g (Marrakchi, Ahmed, Khanday, Asif, &amp; Hameed, 2017)</td>
</tr>
<tr>
<td>Type of FS used</td>
<td>Type of dye used</td>
<td>How the scales were prepared</td>
<td>What were investigated</td>
<td>Characterization</td>
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<td>------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td><em>Labeo rohita</em></td>
<td>Methylene blue (MB)</td>
<td>Carbonized FS by electrical furnace under 99.99% N₂ at 100cm³/min flow rate and 10°C/min heating rate and then washed with NaOH.</td>
<td>Concentration of dye (25-400 mg/L)</td>
<td>BET, SEM, X-ray, FTIR</td>
</tr>
<tr>
<td><em>Oreochromis niloticus</em></td>
<td>Reactive blue 5G dye</td>
<td>Hypochlorite wash, then washing with distilled water, oven dried at 60°C, ground and Tyler sieved of 6, 12, 16, 32, 100, 150 and 325 mesh followed by acid treatment.</td>
<td>Dye Concentration: 100-200mg/L</td>
<td>N₂ physisorption, pH&lt;sub&gt;ZPC&lt;/sub&gt;, SEM-EDX, XRD and FT-IR</td>
</tr>
<tr>
<td>Carp fish</td>
<td>Methyl orange (MO)</td>
<td>Washed with distilled water, decalified with EDTA, sundried and ground.</td>
<td>Dye sol: .03g/L</td>
<td>SEM, FT-IR</td>
</tr>
</tbody>
</table>

Table continued
<table>
<thead>
<tr>
<th>Type of FS used</th>
<th>Type of dye used</th>
<th>How the scales were prepared</th>
<th>What were investigated</th>
<th>Maximum dye adsorption capacity and references</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Tilapia nilotica</em></td>
<td>Ponceau 4R</td>
<td>Washed, sun dried into crispy, taken into NaOH solution and heated in an oil bath, cooled, oven dried at 50°C, ground and sieved through a 160 mesh</td>
<td>Dye concentrations: 50mg/L, Temperatures: 20-25°C, pH:6.5, FS adsorbent doses: 0.08-.2g/L</td>
<td>116mg/g (Zhu et al., 2013)</td>
</tr>
<tr>
<td><em>Dicentrarchus labrax</em></td>
<td>Acid Blue 121 dye</td>
<td>Washed with distilled water, oven dried at 110°C. Extracted hydroxyapatite from scale by alkali and heat treatment.</td>
<td>Contact time: 2h, Dye concentration: 1g/L, Temperature: 30°C, pH:2, Adsorbent ratio: 0.1g/L</td>
<td>298.4mg/g (Uzunoğlu &amp; Özer, 2016)</td>
</tr>
<tr>
<td><em>Leporinus elongatus</em></td>
<td>Remazol Yellow (RY), Blue (RB) &amp; Red (RR)</td>
<td>Washed, immersed into NaOH solution for 4h, washed, dried at 60°C for 6h.</td>
<td>FT-IR</td>
<td>0.325mole/gm (Vieira et al., 2012)</td>
</tr>
</tbody>
</table>

*Table continued*
<table>
<thead>
<tr>
<th>Type of FS used</th>
<th>Type of dye used</th>
<th>How the scales were prepared</th>
<th>What were investigated</th>
<th>Characterization</th>
<th>Process optimization</th>
<th>Evaluation (Equilibrium isothermal, thermodynamic, kinetic, calorimetric and statistical analysis)</th>
<th>Maximum dye adsorption capacity and references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed FS</td>
<td>Acid Blue 113</td>
<td>Washed with hot water and cold deionized water, dried in an oven at 50 °C for 48h, ground in of size between 63 and 100μm, preserved in a desiccator for subsequent experiment.</td>
<td>FTIR, SEM, X-ray, EDX, TGA</td>
<td>Adsorbent dosage: 4 g/L</td>
<td>Initial pH: 6</td>
<td>Langmuir isotherm model: Fitted</td>
<td>145.3–157.3 mg/g (Ooi et al., 2017)</td>
</tr>
<tr>
<td>Oreochromis niloticus and textile auxiliary agents</td>
<td>Reactive blue 5G</td>
<td>Washed with distilled water and oven dried at 60°C.</td>
<td>BJH(^i) and BET</td>
<td>pH: 2</td>
<td>Temperature: 25°C</td>
<td>Langmuir isotherm model: Fitted</td>
<td>291 mg g(^{-1}) (Neves et al., 2017)</td>
</tr>
</tbody>
</table>

BET\(^a\): Brunauer–Emmett–Teller; SEM\(^b\): Scanning Electron Microscopy; TGA\(^c\): Thermogravimetric Analysis; EDX/EDS\(^d\): Energy-dispersive X-ray; FT-IR\(^e\): Fourier-transform infrared spectroscopy; pH\(_{ZPC}\)\(^f\): The pH at point of zero charge; XRD\(^g\): X-ray Diffraction; PSOKM\(^h\): Pseudo Second Order Kinetic model; BJH\(^i\): Barrett, Joyner, and Halenda
2.5 Acid Dyes

The applications of FS for dye removal is mainly for anionic dyes; for example, reactive dyes, acid dyes, azo dyes, sulfur dyes, direct dyes, because the cationic portions of FS attract anionic dyes (Vieira et al., 2012), though Shamik et al. (2012) use the scales of Lebelo rohita for removal of a cationic dye, malachite green. Among the anionic dyes, the reactive dye is mostly used for coloring cellulosic fibers (cotton, rayon etc.). Sulfur and azo dyes have some health hazardous effects that limit their large scale commercial applications (N. N. Mahapatra, 2016). The literature reveals that most of the works are concentrated on the different hues of reactive dyes (Begum & Kabir, 2013; J. Chakraborty et al., 2016; Moura et al., 2012; Ribeiro et al., 2015). Fewer investigations (Uzunoğlu & Özer, 2016), (Shamik Chowdhury et al., 2012), and (Zhu et al., 2013) are carried out on acid, basic and azo dyes. On the other hand, the class of acid dyes is very popular for dyeing natural protein fibers (wool, silk) and synthetic fiber, in particular polyamides (nylons,) as well as acrylics, modacrylics, spandex and their blends. In addition, acid dyes are used in cosmetics, food, and leather industries. That is why the acid dyes require more attention. All types of commercial acid dyes used for textile dyeing are sodium or ammonium salts of sulphonic, carboxylic or phenolic organic acids. Therefore, the acid dyes are readily water-soluble. An acid dye might contain one to four sulphonic groups in their structures, and consequently their molecular weights vary from 300-800 Daltons. During dyeing, the anions of acid dyes attract the cations of fibers and develop strong ionic or covalent bonds with fiber molecules. Acids and salts are used to enhance the dyeing performance by increasing the number of protonated ions of fibers. A palette of colors with moderate to good fastness is available depending on the nature and the size of functional group of dyes.
<table>
<thead>
<tr>
<th>Criteria</th>
<th>Acid red 1</th>
<th>Acid blue 45</th>
<th>Acid yellow 127</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full name</td>
<td>C.I. Acid Red 1, C.I. 118050</td>
<td>C.I. Acid Blue 45, C.I. 63010</td>
<td>C.I. Acid Yellow 127, C.I. 11888</td>
</tr>
<tr>
<td>CAS No.</td>
<td>3734-67-6</td>
<td>2861-02-1</td>
<td>73384-78-8</td>
</tr>
<tr>
<td>M* Formula</td>
<td>$\text{C}<em>{18}\text{H}</em>{13}\text{N}<em>{3}\text{Na}</em>{2}\text{O}<em>{8}\text{S}</em>{2}$</td>
<td>$\text{C}<em>{14}\text{H}</em>{8}\text{N}<em>{2}\text{Na}</em>{2}\text{O}<em>{10}\text{S}</em>{2}$</td>
<td>$\text{C}<em>{26}\text{H}</em>{20}\text{Cl}<em>{2}\text{N}</em>{9}\text{NaO}_{4}\text{S}$</td>
</tr>
<tr>
<td>Chemical structure</td>
<td><img src="image" alt="Single azo class (AR1)" /></td>
<td><img src="image" alt="Anthraquinones (AB45)" /></td>
<td><img src="image" alt="Single azo class (AY127)" /></td>
</tr>
<tr>
<td>M* weight</td>
<td>509.42</td>
<td>474.33</td>
<td>803.63</td>
</tr>
<tr>
<td>Appearance</td>
<td>Red powder</td>
<td>Blue powder</td>
<td>Yellow powder</td>
</tr>
<tr>
<td>Solubility</td>
<td>Soluble in water, fiber element, slightly soluble in ethanol, insoluble in other organic solvents.</td>
<td>Soluble in water, fiber element, slightly soluble in ethanol, insoluble in acetone, benzene, carbon tetrachloride</td>
<td>Soluble in water</td>
</tr>
<tr>
<td>Effect of acid, alkali, and metal ions</td>
<td>Strong sulfuric acid causes light red, and diluted form causes yellow after red; with nitric acid solution, it turns to orange; strong hydrochloric acid results red precipitation, but diluted form gets dissolved. Addition of sodium hydroxide leads to orange brown color. Copper and iron ions in dyeing occasions dark blue color and blue light color, respectively.</td>
<td>The concentrated sulfuric acid turns olive yellow, and diluted form to blue purple. Copper and iron ions lead to dark colored light hair and dark colored light red, respectively.</td>
<td>The strong and diluted sulfuric, nitric and hydrochloric acid gives yellow color, accompanied by precipitation in case sulfuric acid and nitric acid. Diluted sodium hydroxide solution causes yellow precipitation followed by decomposition for thick solution.</td>
</tr>
<tr>
<td>L*</td>
<td>5</td>
<td>5-6</td>
<td>6</td>
</tr>
<tr>
<td>Applications</td>
<td>Good levelness. Mainly used for wool dyeing, polyamide fiber and fabric, silk printing, leather dyeing, also can be used in the manufacture of color amyllum, cosmetics, paper, soap and timber colorants, also used to make the ink</td>
<td>Wool, silk, wool blended fabric, paper, leather, soap and cosmetics shading, its metallic salts used for organic pigment ink</td>
<td>Wool, silk, polyamide fiber fabric dyeing leather dyeing and printing directly</td>
</tr>
</tbody>
</table>

M*=Molecular, L* = Light fastness rating
Taking into account, all these considerations and the absence of sufficient body of knowledge, for our study the anionic dyes presented in Table 2.2, viz., AR1, AB45, and AY127 were used as examples of acid dye series. Reason for considering these three dyes are (1) to study the effect of molecular size, (2) to study the effect of functional groups of dyes on absorption and (3) to compare the results with previous studies, such that on acid blue 121 (Uzunoğlu & Özer, 2016) and acid blue 113 (Ooi et al., 2017). The general properties including structures are presented in Table 2.2.

2.6 Black Drum Fish

In our study, we used the scales of one of the most available fishes in Louisiana: the black drum. Black drum, biologically known as *Pogonias cromis*, having 14 genera, belongs to Sciaenidae family (Clarence P. Luquet et al., 2001). Black drum is commercially and recreationally important fish in the southern part of America especially in Louisiana, Texas, Florida, Mississippi, Virginia, Georgia. Fish processors in Louisiana, Mississippi, and Florida produce more than 3M lbs of fish skins along with scales from black drum and sheepshead during fillet processing (Ogawa et al., 2004; Stunz, 2015). Another study reports that in 2013, the amount of black drum fish harvested solely in Louisiana was over 5M lbs (Davis, West, & Adriance, 2015). While the fishes are processed, the skins along with scales are usually discarded and simply considered waste. Consequently, this waste is a burden for the environment, causes pungent odor and environmental pollution, whereas it could be a good source of biomaterials and bio sorbents (Uzunoğlu & Özer, 2016).

The FS are mainly composed of water (70%), protein (27%), ash (2%), and lipid (1%). Structure of FS consist of 40-90% organic protein matters and 10-60% inorganic mineral substances irrespective to the fish spices (Nagai, Izumi, & Ishii, 2004). The organic portion is
called collagen and the type of collagen found in the black drum is collagen-I due to the presence of high amount of proline, glycine, alanine, hydroxylysine, hydroxyproline. Therefore, amine is the main functional group of collagen (Ogawa et al., 2004). On the other hand, the inorganic portion, calcium-deficient hydroxyapatite, is composed sodium, magnesium, and carbonates attached to phosphate of the hydroxyapatite ions. The appearance of drum’s scales is like Pagrus major, which are orthogonal structure with very low thickness (1-2 micrometer) where the collagen fibers are highly packed in the mineralized hydroxyapatite crystals (Ikoma, Kobayashi, Tanaka, Walsh, & Mann, 2003). Both collagen and hydroxyapatite are responsible for sorption of dye on and into the scales. Collagen adsorbs dyes by electrostatic interaction (chemisorption), absorption, whereas hydroxyapatite does physical sorption, adsorption (Zhu et al., 2013).

2.7 Significance of the Research and Outlining the Research Gap

Louisiana is the greatest water consuming state in the USA for industrial purpose. Louisiana alone used 17% of total industrial water of the USA in 2005 mainly for, chemical, paper industry and petroleum refining (Kenny et al., 2009). USA is one of the largest wool producing and processing countries, and acid dyes are the mostly used dyes for wool strands coloration. Consequently, the wastewater generated by wool colorations need treatments (Jones, 2004). Again, the black drum is one of the most harvested fishes, and the numbers of fish processors in Louisiana are also higher than many other states (Davis et al., 2015; Stunz, 2015). With a view to valorizing fish waste and mutual management of both fish by-product and wastewaters of wool industry, understanding underlying the mechanism of dye removal capacity of drum’s waste might benefit both industries. It is evident that the FS, which are composed of both collagen and hydroxyapatite, are responsible for dye absorption and adsorption respectively. The literature to date investigates the applications of FS and other fish wastes for dye removal through adsorption.
process but not absorption process. Besides, in our study, we attempted to reduce the processes by avoiding extra treatment concerning FS (such as extracting only collages, only hydroxyapatite, chitin, chitosan from skins or scales that have been followed by available research articles for improved adsorption) to approach an easy and largescale treatment of wastewaters. So, the scales, a by-product of fish industry, can be used directly for dye sorption to take the advantages of both collaged and hydroxyapatite, which have not been studied together. In this research, we used direct and powdered scales of black drum to remove dye (AR1, AB45 and AY127) from wool industry wastewaters. The dye absorptions were monitored by US-Vis spectroscopy by studying the concentrations of dye solution before and after the absorption by FS. The results in terms of dye absorption in scales and dye removal percentages were then compared among the used dyes and with the existing studies. The research questions and the research objectives have been summarized below.

2.8 Research Questions

- What is the mechanism of acid dyes absorption into FS?

- What physical and chemical properties are responsible for the dye absorption?

2.9 Objectives

The main purpose of the research is to evaluate the performance of FS, a by-product of fish industry, as an absorbent to treat the wastewaters from textile industry using different acid dyes. Physiochemical and morphological characterization of scales were performed before and after absorption. The absorption process was optimized as function of contact time, dye concentration, absorbent ratio, temperature, pH, and salt concentration etc. Finally, the absorption performance of the proposed absorbent was determined and compared with reported literature.
CHAPTER 3: METHODOLOGY

3.1 Materials and Chemicals

The scales chosen for this project came from the black drum fish, Pogonias Cromis, shown in Figure 3.1 (A). Fish skin along with scales had been collected from a fish processing center, Big D, Franklin, Louisiana (as by-products after removing the fish fillets (Figure 3.2 (B))). The selected acid dyes (acid red 1(AR1), acid blue 45(AB45) and acid yellow(AY127) 127 dyes) were purchased from Crompton & Knowles Corporation, USA, acid (sulfuric acid) from Fischer Scientific, USA, Salt (sodium sulfate) from Mallinckrodt, USA, and all other chemicals such acetone from Sigma-Aldrich, USA.

3.2 Material Preparations

3.2.1 Scales extraction and preparations

According to the owners of the Big D, their average production of FS per week is 10000-20000 lbs, and they usually dispose the scales after separating fish fillets. Ten lbs scales along with skins were taken from Big D. They were then brought to the laboratory and preserved in the refrigerator. The scales were separated, washed thoroughly with water, dried at room temperature and weighed (dried scales) (Figure 3.1 (C)). The scales were ground using Wiley Mill Model 3 (Thomas Scientific, USA), presently housed in the LSU School of Renewable Natural Resources (Figure 3.1 (D)). The powder obtained was fractionated using sieves of different mesh sizes (425, 250, 149, and 75 microns) as shown in Figure (E) and (F). The ground scales bigger than 425 microns were also saved. The crushed as well as non-processed scales were used directly as dye absorbents.
Figure 3.1: Black drum fish (A), scales with skins after separating fish filet (B), scales collected from skins manually (C), Wiley Mill Model 3 (D), FS powder (E) and sieve for screening scales powder of different sizes (F)
3.2.2 Preparation of wastewaters

Stock solutions of 1g/L (0.1%) of chosen dyes were prepared in separate large 1L volumetric flasks. Dye solutions for this research were prepared following a standard wool dyeing procedure followed by LSU Textile Science Laboratory as follows:

- Concentration of dye stock solution: 50, 75, 100, 125, 150 and 200mg/L
- Concentration of salt (Na$_2$SO$_4$): 2.5ml/L (from 25g/L stock solution)
- Concentration of acid (H$_2$SO$_4$): 0.75ml/L (from 10g/L stock solution)
- Material-liquor ratio: 1:40

The pH of the dye solutions were recorded as 2.33 for AB45 and 2.5 for AR1 and AY127. These dye solutions were considered as wastewaters of a wool dyeing practice in which the absorption process of dyes was completed by FS. Treatments had been repeated by changing the dyeing parameters (dye, temperature, pH, and salt concentration, additives) as well as variables for the dye absorption process (contact time, dye concentration, absorbent ratio, vacuum, and stirring) in order to determine their effects on absorption.

3.3 Absorption Process and Analysis of Dye Removal Efficiency

3.3.1 Building of calibration curves for molar absorption of dyes in visible spectrum

Due to their chemical structures (Table 2.2), the concentration of anionic dyes in aqueous solutions can be determined by spectroscopic measurements in the visible spectrum of the light. Calibration curves for all three types of dyes were obtained using five concentrations of dye solution, which were plotted against their corresponding absorption to determine the extinction coefficient ($\varepsilon$) and the molar absorptivity of dyes. The calibration curves representing a linear relationship between concentrations and absorbance were built by preparing several (5) diluted
dye solutions of known concentrations. The wavelengths at which maximum absorbance values of these diluted dye solutions occurred were obtained from UV-Vis (single beam HP8453 diode array spectroscopy, Agilent, USA) spectroscopic measurements (Almquist, Fyda, Godby, & Miller, 2017) as follows: 506nm, 595nm, and 407nm for AR1, AB45, and AY127 anionic dyes, respectively. It is important to note that the blank solvent was the water-acid-salt mixture as in the dye solution. The slopes of the straight calibration lines presented in Figure 3.2 provided the values of extinction coefficients, ε, for each anionic dye: AR1 (ε = 19593 M⁻¹ cm⁻¹), AB45 (ε = 7048.4 M⁻¹ cm⁻¹) and AY127 (ε = 8658.8 M⁻¹ cm⁻¹).

Figure 3.2: Calibration curves of AR1 (A), AB45 (B) and AY127 (C) anionic dye solutions
3.3.2 Absorption process

The wastewaters prepared as per the recipe mentioned in section 4.1.2 were treated considering different process variables such as temperature, absorbent size, absorbent ratio, stirring, acid, salt, acid-salt combination, vacuum and contact time. Practically, dye solution contains salt and acid; however, investigations were also conducted without acid and salt along with different dye concentrations. Dye solutions of a particular concentration (0.05g/L) giving the best absorption results were prepared in a 1L volumetric flask for each dye. In order to maintain consistency, from this dye solution, a 20ml wastewater sample was taken in the treatment bath. Two different temperatures (room and 60°C) were applied to observe the temperature effect, because practically the temperature of wastewater immediately after discharging from the dyeing bath remains high and gradually cools down. Afterward, the absorbent, FS, were added to the dye solutions and the baths were sealed to prevent changing of solution concentration through evaporation. Batch absorption process was applied with continuous stirring for a certain time. Finally, the dye-scale mixtures were centrifuged for five minutes at 4000 RPM using an IEC clinical centrifuge in order to have clear solutions, which were the final solutions after absorption. In another set of experiments, the treatment baths for each dye were kept in vacuum for 1.5 hours after absorption to allow the dye molecules to come in intimate contact of scales, which might provide information whether or not the vacuum has any positive effects. Table 3.1 briefly contains the laboratory experimental conditions for each dye type: AR1, AB45, and AY127.
Table 3.1: Laboratory experimental design and conditions

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Levels</th>
<th>Other process factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyeing variable</td>
<td>Dye concentrations 50, 75, 100, 125, 150 and 200 mg/L</td>
<td>Temperature applied 60°C&lt;br&gt;Time: 1h with continuous stirring at 1000rpm&lt;br&gt;Absorbent size: big powdered scales of 50g/L</td>
</tr>
<tr>
<td>pH</td>
<td>No acid vs. std. Dye solution without acid&lt;br&gt;Temperature applied 60°C&lt;br&gt;Time: 1h with continuous stirring at 1000rpm&lt;br&gt;Absorbent size: big powdered scales of 50g/L</td>
<td></td>
</tr>
<tr>
<td>Salt</td>
<td>No salt vs. std. Dye solution without salt&lt;br&gt;Temperature applied 60°C&lt;br&gt;Time: 1h with continuous stirring at 1000rpm&lt;br&gt;Absorbent size: big powdered scales of 50g/L</td>
<td></td>
</tr>
<tr>
<td>Additives</td>
<td>No salt and acid vs. std. Dye solution without salt and salt&lt;br&gt;Heat up to boil&lt;br&gt;Time: 1h with continuous stirring at 1000rpm&lt;br&gt;Absorbent size: big powdered scales of 50g/L</td>
<td></td>
</tr>
<tr>
<td>Process variables</td>
<td>Scale size Whole scales, and all powdered FS&lt;br&gt;Temperature applied 60°C&lt;br&gt;Time: 1h with continuous stirring at 1000rpm&lt;br&gt;Absorbent size: big powdered scales of 50g/L</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>0.25, 0.5, 1, 1.5, 2, and 2.5 hours&lt;br&gt;Temperature applied 60°C&lt;br&gt;Absorbent size: big powdered scales of 50g/L&lt;br&gt;Continuous stirring at 1000rpm</td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>No temp. vs. std. Time: An hour with continuous stirring at 1000rpm&lt;br&gt;Absorbent size: big powdered scales of 50g/L</td>
<td></td>
</tr>
<tr>
<td>Scale ratio</td>
<td>25, 50, 75, and100g/L&lt;br&gt;Temperature applied 60°C&lt;br&gt;Time: An hour with continuous stirring at 1000rpm&lt;br&gt;Absorbent size: big powdered scales</td>
<td></td>
</tr>
<tr>
<td>Vacuum</td>
<td>1.5 hours vacuum vs. std.&lt;br&gt;Temperature applied 60°C&lt;br&gt;Time: One hour with continuous stirring (1000rpm)&lt;br&gt;Absorbent size: big powdered scales of 50g/L</td>
<td></td>
</tr>
<tr>
<td>Stirring</td>
<td>No stirring vs. std.&lt;br&gt;Temperature applied 60°C&lt;br&gt;Time: One hour&lt;br&gt;Absorbent size: big powdered scales of 50g/L</td>
<td></td>
</tr>
</tbody>
</table>

Std condition: 50g/L dye solution, 2.5ml/L salt, 0.75ml/L acid, 1h time, 50g/L scale (large powdered FS), 1000rpm agitation.
3.3.3 Determination of absorbance of unknown dye concentration

Dye absorption by FS was determined by UV-Vis spectroscopy using the maximum wavelength of absorption of the corresponding dye. The concentration of a solution can be determined by spectroscopic measurements by using the Beer-Lambert law (equation 1).

\[ A = \varepsilon bc \]  

(eq.1)

Where:

- \( A \) is the absorbance, which can be directly read from the spectral data of UV-Vis spectroscopy at particular wavelength,
- \( \varepsilon \) is the molar absorptivity in \( \text{M}^{-1} \text{cm}^{-1} \),
- \( b \) is the path length of the cuvette (usually 1cm), and
- \( c \) is the concentration in mole/L (Calloway, 1997).

The molar absorptivity is obtained for each dye from the respective calibration curves of Figure 3.2 (Rodger, 2013).

3.3.4 Dye removal efficiency

The dye removal efficiency was calculated from equation 2, where \( C_o \) and \( C_e \) are concentration of dye in wastewaters before and after absorption.

\[ \text{Percentage of dye removal} = \frac{(C_o-C_e) \times 100%}{C_o} \]  

(eq. 2)

Also, the amount of absorbed dye by the scales was measured in mg/g at the equilibrium (qe) using equation 3.

\[ q_e = \frac{(C_o-C_e)}{X_o} \]  

(eq. 3)

Here, \( C_o \) and \( C_e \) are the initial and final dye concentration in mg/L, and \( X_o \) is the amount of absorbent used in the absorption process in g/L (Uzunoğlu & Özer, 2016).
3.4 Physiochemical Analysis

All the physiochemical analysis (except TGA) were performed before and after dye absorption by the scales. In the case of colored scales after absorption, only acid blue 45-absorbed scales were used for the following analyses.

3.4.1 Morphological analysis

The superficial structure and morphology of the scales were unveiled at different magnifying levels (100X, 1000X and 5000X) by scanning electron microscopy, SEM, using a JSM-6610LV instrument (JEOL, Japan) in low vacuum at 15Kv (Ribeiro et al., 2015). Zeiss Lumera Light Microscope (Carl-Zeiss, Germany) provided 6.4X images of both sides of the whole scale.

The elements present in the FS were detected by Energy Dispersive X-ray Spectroscopy, EDS (EDAZ AMETEK material analysis division, USA), usually coupled with the SEM system (Ooi et al., 2017). Before SEM-EDS analysis, the samples (colored and uncolored) were coated with a thin layer of gold using an EMS550X Sputter Coater (Electron Microscopy Sciences, USA).

3.4.2 Thermal analysis

Thermogravimetric analysis (TGA) was performed to determine the thermal behavior, crystallinity, and elemental composition of FS. For TGA analysis, 2.03mg FS was taken in an aluminum pan heated under nitrogen atmosphere at a heating rate of 10°C/ min from room temperature (RT) to 600°C using AutoTGA 2950HR V5.4A (Thermal Instruments, USA).
3.4.3 Chemical analysis

Fourier transform infrared, FT-IR, spectroscopy analysis was performed in order to disclose functional groups (Bhuiyan et al., 2018) present in the pristine scales, powdered scales, dyes (AB45), and colored scales between 400 and 4000cm⁻¹ wavenumber using a Bruker Alpha & Tensor 27 FT-IR spectrophotometer and OPUS software (USA). This instrument requires no sample preparation.

3.5 Statistical Analysis

A two-way analysis of variance (ANOVA) at 5% level of significance using SAS 9.4 was conducted to investigate whether or not there are any significant differences in the percentages of dye removal among different dye types (AR1, Ab45 and AY127), among different process parameters (temperature, salt, acid, additives (salt and temperature together) stirring and vacuum) and among ‘die 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 test of 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 control process and one for the other treatment levels.

3.6 Rheology of the Jellified Wastewater (Gel)

An astounding observation was found after the dye absorption by FS, which was the jellification of the wastewater (gel formation); this phenomenon occurred using all three dye types. The gel was obtained by ‘aging’ of the solution used for coloration of FS (containing sodium sulfate, sulfuric acid and the blue dye) after removing of scale particles by centrifugation. To manage the disposal of the final jellified wastewaters after the absorption by scales, this research
was extended in order to understand the behavior of the gel; therefore, the gel (blue) was characterized by heating and cooling temperature ramp experiments with using a TA 1000 Rheometer (Thermal Instruments, USA). The following parameters have been employed:

- Temperature range, 15-80°C heating (85-10°C cooling)
- Temperature step, 5°C/minute
- Frequency, 0.6283 rad/sec
- Oscillation Stress, 0.7958 Pa
- Parallel plate geometry (steel plates 40 mm diameter)
- Gap, 300 microns

The solution contained 2.5g/L sodium sulfate + 0.75g/L sulfuric acid (taken from their stock solutions), 0.05g/L and 0.41% collagen. The gelatin content was calculated as the difference between the percentage of solids obtained by evaporation (drying) of liquids containing sodium sulfate, sulfuric acid and dye before and after adding the scales for coloration. Aging refers to the time in which a solid gel was formed by standing at room temperature for 24 hours.

All the test results provide detailed and comprehensive information about the absorption behavior of acid dyes into FS. Test results have been compared with other research findings in the following chapter. The absorption process and the rheology were conducted in LSU Textile Science Lab. Other chemical analysis such as SEM-EDS, EMS550X Sputter Coater, FTIR were investigated using LSU Shared Instrument Facility (SIF), and TGA from the Polymer Analysis Laboratory from the LSU Department of Chemistry.
CHAPTER 4: RESULTS AND DISCUSSION

4.1 Characterization

4.1.1 Drum’s scale architecture

The exquisite structure of the surface topology of drum’s scale belongs to disk-like cycloid typed scale (Lagler, 1947). Scale protects the body of fish from any unexpected attack or accident. Figure 4.1 shows 6.4X images of both sides of a scale before and after dye absorption. Although they look the same, they are actually different. Figure 4.1 (A) and (B) show the rough (top) and smooth (bottom) sides of the FS, respectively. The side that remains in contact to the body is smoother and contains jelly like organic polymer called collagen (Zhu et al., 2013). No variation in the surface view of the smoother side is noticeable even in 100 (100µm), 1000 (10 µm) and 5000 (5µm) times magnified SEM snaps presented in Figure 4.1 (B). On the other hand, the outer side (rough side) as shown in Figure 4.1 (A), comprised of mostly inorganic contents (as revealed from EDS analysis), is relatively rough and with different architectures. From the structural viewpoint, it has four major segments marked with number 1, 2, 3 and 4. The center of the scale marked with number 2 is called focus, having beautiful mosaic architecture; it is hard due to high inorganic crystal matter referred as apatite. The front part marked with 1, stair like ridges separated by radii and secondary radii (white inflexion lines), is known as the anterior field. The other side assigned by number 3, called posterior field decorated with meander lined tiles, is the part that is embedded within the fish body. The other two sides are designed by arc type lines called circuli, which are divided from the posterior field by lateral fields (between number 3 and 4) in both the upper right and the lower right corners (highlighted with red ellipses) (Lagler, 1947).
After the dye absorption, it is evident that the shade depth on either side of the scale is different. Since the dye absorption by FS is due to the electrostatic attraction between the positively charged fish protein groups (mainly amine) and the anionic part of the acid dyes, it might be considered that the side containing high collagen content should take more dye compared to the inorganic side (Zhu et al., 2013), fact which is obvious from Figure 4.1 (C and D) as well. The images of pristine rough (top) and bottom (smooth) sides of scale (Figure 4.1 (A and B)) are from same scale and colored ones (Figure 4.1 (C and D)) from same scale, and they were taken under the same light source using the Zeiss Lumera Microscope.
4.1.2 SEM and EDS

The surface morphology of the FS was examined on both sides before and after the dye absorption through SEM at 1000X magnification. Figure 4.2 (A and B) shows images of circuli (the outer scale side) before and after dye absorption, respectively. Figure 4.2 (C and D) represents the same for the inner side (smoother). No significant differences have been perceived between the images of same unit. A similar observation has been noticed by Uzunoğlu and Özer (2016) in their research. However, in Figure 4.2 (A and B), some cracks are visible, and the cracks in the dye absorbed FS (B) are somewhat smaller than that of the pristine FS image (A); it might be an indication of dye anchoring, but not as obvious as noted by Zhu et al (2013). The reason of significant finding reported by these authors is due to some chemical treatments with NaOH followed by heat application in order to increase the pore size of the FS so that the dye adsorption can be maximized. Ribeiro et al. (2015) diagnosed the untreated FS surfaces as non-porous, having therefore a poor adsorption. In the present study, no chemical treatment or external heating were applied, since they involve extra cost and process complexity.

![Figure 4.2: SEM images of the pristine rough side of FS before (A) and after (B) dye absorption, and of the smooth side of FS before (C) and after (D) dye absorption](image-url)
Table 4.1: EDS elemental analysis (expressed in percentage of weight) and comparisons with other reported findings

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Elements</th>
<th>C</th>
<th>N</th>
<th>O</th>
<th>Na</th>
<th>Mg</th>
<th>P</th>
<th>S</th>
<th>Ca</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drum</td>
<td>P*</td>
<td>27.02</td>
<td>16.65</td>
<td>38.62</td>
<td>0.7</td>
<td>0.53</td>
<td>7.25</td>
<td>9.22</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R*</td>
<td>55.41</td>
<td>23.49</td>
<td>21.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>48.58</td>
<td>18.72</td>
<td>32.72</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rohita</td>
<td></td>
<td>46.08</td>
<td>26.64</td>
<td>24.86</td>
<td>0.91</td>
<td>0.24</td>
<td>1.27</td>
<td></td>
<td>(Marrakchi, Ahmed, et al., 2017)</td>
<td></td>
</tr>
<tr>
<td>Tilapia</td>
<td></td>
<td>45.7</td>
<td>38.9</td>
<td>0.2</td>
<td>0.2</td>
<td>5.6</td>
<td>9.4</td>
<td></td>
<td>(Ribeiro et al., 2015)</td>
<td></td>
</tr>
<tr>
<td>E* bass</td>
<td></td>
<td>40.1</td>
<td>0.54</td>
<td>24.0</td>
<td>8.47</td>
<td></td>
<td>9.6</td>
<td></td>
<td>(Uzunoğlu &amp; Özer, 2016)</td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td></td>
<td>33.76</td>
<td>38.81</td>
<td>7.97</td>
<td>19.01</td>
<td></td>
<td></td>
<td></td>
<td>(Ooi et al., 2017)</td>
<td></td>
</tr>
</tbody>
</table>

P*=Pristine FS, C*=Colored FS, R*=Rough surface of FS, S*=Smooth surface of FS, E*=European

EDS elemental analysis identifies and quantifies elements present in a material. It confirms the presence in FS of C, O, N, Na, Mg, P, S, and Ca and their weight percentages are listed in Table 4.1. The data reinforce the differences in the percentage of weight content in different places of FS, although all studies consulted in this paradigm have concurred that the enlisted elements are the full elemental profile of fish scales (Marrakchi, Ahmed, et al., 2017; Ooi et al., 2017; Ribeiro et al., 2015; Uzunoğlu & Özer, 2016). Uzunoglu and A. Ozer (2016) noted the same regarding various amounts of elements and commented that the heterogeneous structure of the fish scale is the reason behind it. The inner side of the scale is made of organic matters as it contains high amounts of C, O and N. In contrast, in the outer side, the presence of Ca, S, P along with some organic matters and Ca:P ratio (1.27 and 2.14 before and after dye absorption, respectively) affirms the presence of inorganic compounds such as hydroxyapatite, Ca_{10}(PO_4)_6(OH)_2, and/or carbonated apatite, Ca_{10}(PO_4)_6CO_3 (Ribeiro et al., 2015). The reason of
positive change in the Ca:P ratio (from 1.27 to 2.14) might be the leaching of the organic matter as a result of the temperature application during the dye absorption process, which might cause resultant increment of other elements. Although, Ribeiro et al (2015) pointed out that the leaching of inorganic substances occurs in acidic medium, the gelatin formation in this study supports leaching of organic matters. The same notion (changing C, N and O percentage) is also seen from smoother surface of the FS.

4.1.3 Thermo-gravimetric analysis of FS

Thermal degradation of the FS in terms of weight loss percentage as a function of temperature was investigated by thermo-gravimetric analysis (TGA) and the results are illustrated in Figure 4.3. The initial weight loss is accounted as 14.2% between 25°C and 150°C as a result of evaporation of adsorbed water (to 110°C) and of the hydrogen-bonded (absorbed) water (to 150°C). The subsequent weight loss (about 3%) occurring to 246°C might be due to removal of remnant water from the inorganic lattice structure and decomposition of light volatile compounds in FS (Ooi et al., 2017). The thermal decomposition temperature is set at 285°C, with the maximum loss of weight (almost 0.2%/°C) at 332.5°C. In the temperature domain of 285°C to 400°C, Torres, Troncoso, & Amaya (2012) assumed that collagen polymeric chains might melt, degrade and decompose. Decomposition of other macromolecular substances and evolution of gases take place after 400°C and finally result about 50% residues of inorganic apatite by losing 50% weight from its initial status (R. Chakraborty, Bepari, & Banerjee, 2011; Mota, Silva, Vieira, Sussuchi, & Cestari, 2013). According to Ooi et al (2017)’s observation, FS has significant amounts of volatile compounds and low ash content. Marrakchi et al (2017) obtained a 12.18% residue by carbonizing rohita fish scales at 600°C temperature.
4.1.4 FT-IR

The Fourier-transform infrared spectra (FT-IR) of raw scales (as such) and of pulverized scales present the absorptions characteristic to peptide (amide) groups from polymer chain amino-acids, -NH-C(=O)-, labelled as Amide A, Amide I and Amide II, together with the bands typical to absorbances for phosphate and carbonate ions in the apatite lattice containing hydroxyapatite, \( \text{Ca}_{10} \text{(PO}_4\text{)}_6 \text{(OH)}_2 \), and calcium carbonate, \( \text{CaCO}_3 \), are presented in Figure 4.4 (A). However, as shown in Figure 4.4 (B), after grinding to 75\( \mu \), 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).
This is an important observation, because the apatite components are the major players in reacting with sodium salts of sulfonic acid dyes. After dyeing, the Amide I and Amide II absorptions are present both in raw (as such) and in colored scales, while the phosphate and carbonate anions are absent because of their migration in water as sodium soluble salts. An example is given in Figure 4.5 which compares 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 4.5 (A)), while this spectrum is lacking \(-\text{PO}_4\) and \(-\text{CO}_3\) anion bands (Figure 4.5 (B)).
4.2 Effects of Process Parameters

4.2.1 Effect of the initial dye concentration and of the amount of FS used

Several dye stock solutions, viz., 50-200mg/L, were prepared to investigate the effect of the initial dye concentration. Figure 4.6 (A) presents the relationship between the initial dye concentration and the dye removal percentage. No significant change has been noticed in the dye removal percentage with the increment of the initial dye concentration; however, the dye absorption of FS increases linearly for all three dyes (acid red 1, acid blue 45 and acid yellow 127). The binding sites of FS continuously absorb dye molecules in their active binding sites, and because the availability of dyes increases with the dye concentration, the overall dye removal percentages remain almost constant; this will cause more dye loading on FS, and therefore the dye-uptake increases. The dye removal percentages for AR1, AB45 and AY127 are around 44%, 64% and 84%, and maximum dye loadings on FS for those dyes are 1.8, 2.7 and 3.4mg/g, respectively. On the other hand, dye removal percentages increase and tend to attain equilibrium at the end of the curves with the increase of the scale amount from 25 to 100g/L, followed by gradual diminution of absorption for constant dye concentration (50mg/L), since there are few transferrable dye molecules available, compared to scale quantity (Figure 4.6 (B)). Researchers found different results while examining those effects (dye concentration and amount of scales used); for instance, Marrakchi et al (2017) noted a similar observation in their findings regarding the removal of reactive orange 16 and methylene blue dyes by carbonized FS. In a nutshell, concentration difference in both dyes and scale amounts is a driving force that causes mass transportation between sorbents and sorbates, and defines the direction of dye molecules to move depending on the attainment of equilibrium, dye aggregation and saturation point, which, however, might be influenced by other process variables such as time, temperature, salinity, pH, and
surfactant, if used (J. Chakraborty et al., 2016; Shamik Chowdhury et al., 2012; Marrakchi, Ahmed, et al., 2017; Marrakchi, Auta, et al., 2017; Ooi et al., 2017).

![Figure 4.6: Effect of initial dye concentration (A) and amounts of scale used (B)](image)

Table 4.3 offers a comparison of dye removal capacities of different dyes by different FS along with other adsorbents, which shows the lower performance of our FS than many other FS sorbents. Several reasons could explain this difference in results obtained. Firstly, the dyes and FS used in this study are different from the reported literature. Table 4.3 shows the varied results for different dyes for same type of FS. Our approach is based on the absorption process rather adsorption, which we didn’t find discussed in literature consulted. Therefore, the performance achieved in the present study and other adsorption processes should not be compared. Secondly, the absorption process is triggered by electrostatic attraction between sorbents and sorbates and causes penetration of sorbate molecules into sorbents. On the other hand, adsorption being a physical process is the surface aggregation of dye molecules (Gupta et al., 2009). Hence, the absorption capacity depends on the number and amount of dye binding functional groups in the sorbent material. The organic FS content, mostly collagen, which has the dye binding groups, has been noted between 40-90%, depending on the fish species (Nagai et al., 2004). The high content
of Ca and P in our FS, as well as the Ca:P ratio obtained from EDS analysis, confirms the presence of high inorganic material in drum’s scales, which might lead to a low dye pick-up by overall collagen. Thirdly, we used significantly high amount of FS (25 to 100g/L for investigating the effect of scale amounts, and 50g/L for all other process variables), whereas none of the studies mentioned in Table 2.1 used over 4g/L. In our approach, we did not process FS, so it contained high moisture and volatile matters, which is also evident from TGA analysis. Again, the gel formation after the dye removal due to the leaching of some organic matters leads to depletion of dye binding collagen sites in FS. Thus, the actual amount of dye absorbing collagen was not much compared to the whole amount of scale used. Consequently, a high amount of scales, i.e. high denominator value ($X_o$) of equation 3, gives a low $q_e$ value, dye absorption by sorbent (FS). Finally, most of the studies cited above processed FS through heat and/or chemical treatment that resulted in moisture and volatile evaporations; for instance, Ooi et al (2017) noticed that application of high heat, such as 800°C, ended up with 8.6% ash content, and Marrakchi et al (2017) yielded 12.18g carbonized FS out of 100g by heating at 600°C. Ultimately, these processes come up with high removal performance, since the density gets lower (the volume remains almost the same but the weight decreases significantly). However, the dye removal percentage in this study is quite satisfactory and effective for low dye concentration of acid dyes. The variations in dye removal performances for different dyes used in this reseach deserve explanation, which might be due to bulky chemical structures and size of dyes. Although, AY127 has a higher molecular weight (803.63) than AR1 (509.42) and AB4 (474.33), its linear structure enables an easy penetration in FS if compared to that of the bulky structures of other two dyes. Different absorption of dyes might be due to the difference in their structures. Presence of chemical functionalities which participate absorption process, makes the differences in the amount of absorption.
Table 4.2: Comparison of dye binding by FS and other adsorbents

<table>
<thead>
<tr>
<th>Sorbent types</th>
<th>Sorbates</th>
<th>qe in mg/g</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated carbon</td>
<td>Reactive blue 5G</td>
<td>149.13</td>
<td>(Martins, Schimmel, dos Santos, &amp; da Silva, 2012)</td>
</tr>
<tr>
<td>Activated carbon</td>
<td>Blue 5G</td>
<td>0.88</td>
<td>(Y. Al-Degs, Khraisheh, Allen, &amp; Ahmad, 2009)</td>
</tr>
<tr>
<td>Palm bark</td>
<td>methylene blue.</td>
<td>2.66</td>
<td>(Sun, Wan, &amp; Luo, 2013)</td>
</tr>
<tr>
<td>Eucalyptus</td>
<td>methylene blue.</td>
<td>2.06</td>
<td>(Sun et al., 2013)</td>
</tr>
<tr>
<td>Tilapia nilotica</td>
<td>Se</td>
<td>1.02</td>
<td>(Kongsri, Janpradit, Buapa, Techawongstien, &amp; Chanthai, 2013)</td>
</tr>
<tr>
<td>Labeo rohita</td>
<td>Brilliant Red (C. I. Reactive Red 2)</td>
<td>3.19</td>
<td>(Begum &amp; Kabir, 2013)</td>
</tr>
<tr>
<td>Labeo rohita</td>
<td>Malachite Green</td>
<td>38.46</td>
<td>(Shamik Chowdhury et al., 2012)</td>
</tr>
<tr>
<td>Labeo rohita</td>
<td>Reactive orange 16</td>
<td>114.2</td>
<td>(Marrakchi, Ahmed, et al., 2017)</td>
</tr>
<tr>
<td>Labeo rohita</td>
<td>Methylene blue (MB)</td>
<td>184.40</td>
<td>(Marrakchi, Auta, et al., 2017)</td>
</tr>
<tr>
<td>Oreochromis niloticus</td>
<td>Reactive blue 5G dye</td>
<td>241.2</td>
<td>(Ribeiro et al., 2015)</td>
</tr>
<tr>
<td>Tilapia fish</td>
<td>Ponceau 4R</td>
<td>116</td>
<td>(Zhu et al., 2013)</td>
</tr>
<tr>
<td>Dicentrarchus labrax</td>
<td>Acid Blue 121 dye</td>
<td>298.4</td>
<td>(Uzunoğlu &amp; Özer, 2016)</td>
</tr>
<tr>
<td>Leporinus elongatus</td>
<td>Remazol Yellow (RY), Blue (RB) &amp; Red (RR)</td>
<td>217.4</td>
<td>(Vieira et al., 2012)</td>
</tr>
<tr>
<td>Mixed FS</td>
<td>Acid Blue 113</td>
<td>145.3–157.3</td>
<td>(Ooi et al., 2017)</td>
</tr>
<tr>
<td>Oreochromis niloticus</td>
<td>Reactive blue 5G</td>
<td>291</td>
<td>(Neves et al., 2017)</td>
</tr>
<tr>
<td>Pogonias cromis</td>
<td>Ara, AB45, AY127</td>
<td>1.8, 2.7 and 3.4 respectively</td>
<td>This study</td>
</tr>
</tbody>
</table>

qe= Amount of dye absorbed/adsorbed in sorbets

4.2.2 Effect of scale size and contact time on acid dye removal

Scale sizes and the contact time have considerable influence on the dye removal percentage. Experiments were conducted using FS powder of different sizes, such as 75, 149, 250, 425 microns and larger, as well as pristine whole scales. Different contact times (0.25, 0.5, 1, 1.5, 2 and 2.5h) were applied when large powdered FS were used. Figure 4.8 (A) explains that the dye removal efficiency is positively correlated with the size of FS, and a typical picture of this outcome
has been shown in Figure 4.7, which contradicts previous findings for the adsorption process. As the adsorption process is subjected to the surface area of sorbents and the distribution of pores on the surface, making fine powder out of whole scale improves specific surface area of FS and therefore the potential to accumulates more dye molecules (Iqbal et al., 2011). On the contrary, absorption caused by electrostatic attraction does not depend upon the surface area but on the availability of active dye binding sites. Besides, the scales were washed thoroughly with water after the discarding of wastewaters, without any dye coming out, confirming therefore the fixation of dye molecules (by absorption) into FS.

The cationic collagen’s functional groups attract the anionic acid dyes immediately after they come in contact, and mass transfer of dye molecules from aqueous solution to sorbent occurs rapidly. Consequently, as depicted in Figure 4.8 (B), the removal percentage of the dye is significantly high in the first 30 minutes; after an hour it becomes constant, indicating the achievement of equilibrium (Shamik Chowdhury et al., 2012). Most of the consulted studies (Begum & Kabir, 2013; Marrakchi, Ahmed, et al., 2017; Marrakchi, Auta, et al., 2017; Ribeiro et al., 2015; Uzunoğlu & Özer, 2016; Zhu et al., 2013) reported the equilibrium time at 2h or even after that time span, except for findings of Ooi et al’s (2017) (15-20mins) and Begum & Kabir’s (2013) (30mins). Therefore, lower eqilibrium time and better absorption capacity for whole scales suggest that our proposed process is time saving and cost effective. Moreover, these dyed scales can be used later for an ornamentation purpose, such as designing decorated furnitures, upholstry and apparels. Meanwhile, the blue FS are being used by an apparel design professor for adorning her works to be submitted to the coming 2018 International Textile and Americal Association (ITAA) conference. The effects of both time and scale sizes for dye removal were consistent through all three anionic dyes.
Figure 4. 7: Effect of the particle size of ground FS on the absorption of anionic blue dye: Absorbed dye in scales from whole to fine powered (75, 149, 250, 425µ, > 425µ ) FS (left to right) (A); Sample wastewater after treatment by FS of different sizes (whole to fine powder, left to right) (B). Process time: 1h and temperature: 60°C.

Figure 4. 8: Effect on dye removal of scale size (A) and of contact time (B). Process time: 1h and temperature: 60°C.
4.2.3 Effects of temperature, salt, acid, additives (acid and salt), stirring, and vacuum on anionic dye removal

In our experimental design, sample wastewaters were prepared following standard recipe for wool dyeing: 50mg/L dye, 2.5g/L salt (Na2SO4) from 25g/L stock solution, 0.75g/L acid (H2SO4) from 10g/L stock solution. Practically, for dyeing wool fabrics, the temperature of the bath is raised to boil, and after dyeing, during discharging, the wastewaters were let to cool down to 60°C. Thus, the wastewater containing acid and salt as indicated above, with the dye absorption process running at 60°C with constant stirring (1000 rpm) for one hour define the standard parameters, which were applied throughout all experiments. Here, the effects of those variables mean that particular variable was omitted from standard dye solution and process conditions (since standard dye solutions have all process parameters of actual wool dyeing solution). An additional experiment was conducted to look into the effect of vacuum on absorption of anionic dyes by FS. Table 4.3 summarizes the conditions of different process for the study of the effects temperature, salt, acid, additives (acid and salt), stirring, and vacuum.

Table 4.3: Summary of process conditions

<table>
<thead>
<tr>
<th>ID for statistical analysis</th>
<th>Treatment effects</th>
<th>Parameter applied (√ mark means applied, - if not)</th>
<th>Vacuum for 1.5h after absorption process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Std.</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>AR1/AB45/AY127 2</td>
<td>No temp</td>
<td>-</td>
<td>√</td>
</tr>
<tr>
<td>AR1/AB45/AY127 3</td>
<td>No salt</td>
<td>√</td>
<td>-</td>
</tr>
<tr>
<td>AR1/AB45/AY127 4</td>
<td>No acid</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>AR1/AB45/AY127 5</td>
<td>No additives</td>
<td>√</td>
<td>-</td>
</tr>
<tr>
<td>AR1/AB45/AY127 6</td>
<td>No Stirring</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>AR1/AB45/AY127 7</td>
<td>Vacuum eff</td>
<td>√</td>
<td>√</td>
</tr>
</tbody>
</table>

Process time: 1h
Figure 4.9: Effect of other process parameters

Figure 4.9 represents the effects of all those variables across all dyes are consistent except in the case of temperature for AY127 dye. It delineates increased temperature improves the absorption performance, meaning that the absorption process is endothermic (Y. S. Al-Degs, El-Barghouthi, El-Sheikh, & Walker, 2008; Shamik Chowdhury et al., 2012), whereas except Chowdhury et al., all other researchers found that their adsorption processes by FS were exothermic. Temperature swells and increases the pore size of the material so that dye molecules can easily penetrate the sorbent system (N. Mahapatra, 2016). Besides, the temperature increases mobility of dye molecules and reduces repulsive forces acting on them, which might improve the dye binding capacities of FS (Shamik Chowdhury et al., 2012).
The additives (salt and acid) negatively affected the dye removal efficacy. Although practically dye solution for wool dyeing contains those additives (salt and acid), experiments were conducted to know how the removal performance without additives was. Consequently, dye solutions without acid and salt were prepared, and percentage of removal were investigated and compared to the standard wastewater sample. From Figure 4.9, the performance is highly significant in the case of dye solution without any additives for all three dyes: AR1 (44 to 64%), AB45 (65 to 89%) and AY127 (79 to 92%). Figure 4.10 shows wastewater samples these solutions before and dye absorption by FS, which provided the highest percentage of removal for all three dyes. Occupying some active dye binding sites of FS by additives (salt and/or acid or their combination) bring about these results. The effect of pH meaning wastewaters containing acid (pH = 2.33 for AB45 and pH=2.5 for AR1 and AY127) but no salt enhances dye up-take. Acidic solution assist FS to be positively charged; therefore, they can attract the negatively charged anionic dyes. In fact, at lower pH, FS along with collagen containing positively charged amine (-NH₃⁺) groups becomes more positive to draw negatively charged sulfonate (–SO₃⁻) groups of dyes; and at higher pH, FS being negatively charged repulse dye molecules (Begum & Kabir, 2013; Ooi et al., 2017). On the other hand, presence of salt but no acid negatively affects the process performance implying that probably salt is the one, which might engage with some dye binding
sites. Chakraborty et al (2016) find no significant effect of salt addition (NaCl) in their study regarding carp FS adsorbent for methyle orange dye removal.

Figure 4.9 also reveals notable positive effect of stirring and a slight negative for vacuum. Stirring boosts up dye removal percentage considerably, because it helps reduce the diffusional and hydrodynamic boundary layers on FS, which resist the mass transferring process from aqueous solution to the absorbent (Adeyemo, Adeoye, & Bello, 2017; Moreira, Peruch, & Kuhnen, 1998). For each dye type, treatment baths were kept in vacuum for 1.5 hours after having completed the usual absorption process to allow the dye molecules to come in intimate contact with scales. However, the experiments came up with slightly discouraging outcomes.

4.3 Statistical Analysis

The effects of different dye types (AR1, AB45, and AY127) and different process parameters (temperature, salt, acid, additives (acid and salt), stirring, and vacuum) including ‘die type X process’ interactions on the dye removal% were analyzed by two-way ANOVA at 5% level of significance using SAS 9.4 program. The results showed that all the main effects (dye type and process parameters) and their interactions (dye type X process) were statistically significant meaning that all the factors including dye type, process and ‘dye type X process’ interactions have significant effects to the model. The population least square means for each of the interaction combination were also found significantly different from 0. Additionally, “slice” option is used to test the effects of ‘dye type X process’ within each type of acid dyes, which showed that within each type of acid dyes, the effects of 7 dye absorption processes are significantly different.
Figure 4.11: Pairwise comparisons between the control and the other processes within each type, acid red 1 (AR1) (A), acid blue 45 (AB45) (B) and acid yellow 127 (AY127) (C)
Apart from the testing effects, Dunnett’s pairwise comparisons between the control and the other processes within each type of acid dyes have been summarized in the above illustrations with 95% decision limit (Figure: 11 (A), (B) and (C)), which states (1) for acid red 1 (AR1), vacuum and no acid treatments made no significant difference on the dye removal% when comparing with standard process (2) for acid blue 45 (AB45), there was no significant difference for the process without acid when comparing with standard process and (3) for acid yellow 127 (AY127), there were no significant differences for the processes without salt and acid when comparing with standard process. It also reveals that application of temperature and stirring had significant positive effect on the improvement dye removal%, which is 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 is not statistically significant for AY127; on the other hand, application of vacuum showed negative effect for all dyes; nonetheless, it is not significant for AR1. The presence of salt (no acid treatment level) had slight negative effect; however, no statistically significant deviations were found for all dye types while comparing to the control group. The results might not be much reliable, because there is no sufficient replication for the response variable.

4.4 Rheology of the Jellified Wastewater (Gel)

The protein component of scales (collagen) is extracted in the acidified dye bath due both to temperature and salt. The presence of the acid dye as a water solution which in time is transformed to a gel at room temperature as shown in Figure 4.12. For the blue gel the rheology measurements have shown that both the elastic component (G’) of the gel rigidity (G*) and the viscosity increased first with temperature when the system becomes non-homogeneous in initial
stages of melting, with a rapid collapse after gel melting at around 30°C (Figure 1 (A) and Figure 4.14 (A) respectively).

Figure 4. 12: Gel formation

Figure 4. 13: Variation of the elastic component $G'$ of the blue gel in heating (A) and cooling (B) experiments.

A reverse scenario was noticed by cooling the collagen solution back room temperature, in which $G'$ of the collagen solution increased as the temperature went down to 60°C, decreased due to the non-homogeneity of the system in the temperature range preceding total jellification (around 30°C again), and increased after that in a normal way, i.e., $G'$ of a homogeneous system increases as temperature decreases. One should notice the scale of $G'$ variation from a firm gel (>100 Pa) to a liquid (Figure 4.13 (A)) and vice-versa to a weak gel (<10 Pa) as presented in Figure 4.13 (B). Similar scale reduction was observed for the viscosity of the
gel from >50 Pa.s before melting (Figure 4.14 (A), heating run) to <1 Pa.s of the week gel formed in the cooling run (Figure 4.14 (B)).

Figure 4.14: Variation of the viscosity of the blue gel in heating (A) and cooling (B) experiments.

The results of these rheological measurements have a practical application for handling of wastewaters of wool dyeing with acid dyes after removal of remnant dyes using fish scales. To our best knowledge the rheology of these wastewaters obtained after removing of fish scales has not been yet investigated. The values of elastic component (G’) of the gel rigidity (G*) and the viscosity (η) were directly obtained from the curve generated by rheometer.
CHAPTER 5: CONCLUSIONS, LIMITATIONS AND RECOMMENDATIONS

In the present study, the suitability of fish scales (FS) as a prospective bio-sorbent was assessed by different chemical analyses including SEM-EDS, TGA, XRD, XRF and FT-IR. Process performance in terms of percentage of dye removal and dye absorption along with their optimizations was carried out by investigating different process variables such as dye and FS initial concentrations, contact time, FS size, temperature, pH, salt, combination of salt and pH, stirring and vacuum using UV-Vis spectroscopy.

SEM and EDS unveiled the structural and chemical variations of FS, whereas TGA identified approximately 50% moisture, organic and volatile components and 50% mineral content in FS structure. FT-IR spectra confirmed the presence of the dye in the dyed FS.

Dye absorption increased with increasing initial dye concentration, amount of scale used and scale sizes and decreased with incorporation of additives (salt and acid), particularly salt. Best removal percentages were found for dye solution without any additives. Contact temperature and time having slight positive effects provided that the absorption processes were endothermic and achieved equilibrium after an hour. Stirring had significant effect on removal capacities of FS, but vacuum impacted it negatively. Two-way ANOVA showed the dye removal% of three dyes were significantly different. The effect of process parameters and the ‘dye types X process’ interaction had also significant effect on the dye removal performance.

Finally, an amazing observation was noticed when the wastewaters after absorption and subsequent centrifugation process were allowed to stay for a long time like 24 hours that they turned into gel, which was only found in the case of wastewater sample having acid and salt followed FS absorption process with big scales especially whole and large powdered scales.
According to our findings, big size scales and wastewater without any additives showing best performance implies an obvious indication of saving process and chemical cost. The colored FS could be used for many functional, ornamental and design applications.

This study was conducted only using FS of black drum, more fishes can be considered for future study. Our research was in laboratory scale, pilot scale can be conducted to approach practical scenario. To the best of our knowledge, dye removal through absorption was not addressed before, and this study was the first attempt of its kind. In the present study, we didn’t study surface of FS in terms of surface specific area, porosity, pore diameter and total pore volume. As a result, more research should be performed in order to optimize the dye absorption and dye adsorption processes, such as determination of pore size of FS in order to correlate the surface area of FS to efficiency of dye removal.

Future studies can be conducted to investigate the underlaying chemistry of gel formation and properties of the jellified wastewaters. The jellified protein (collagen), processed as thin films, might find some promising applications in medical and biomedical (protection, drug delivery), cosmetic (micro- and nano-scale protein ointments) and food (micro packaging) industries.

The whole fish skin containing both the FS and some meat rejected by processing the fish by filleting, might be used after drying to enhance both the performance of dye absorption and the economics of the wastewater treatments. The protein/inorganic complex should be tested also for removing of unwanted materials from other wastewaters, such the removal of toxic and heavy metals (Mercury, Chromium, Uranium, etc.) from particular industries. Additionally, due the high content of inorganics (hydroxyapatite and carbonates), FS will be a good filler for fabrication of polymer composites, such as PVC/FS.
REFERENCES


VITA

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