Preparation, characterization of fish scales biochar and their applications in the removal of anionic indigo carmine dye from aqueous solutions

George O. Achieng, Chrispin O. Kowenje, Joseph O. Lalah and Stephen O. Ojwach

ABSTRACT

The preparation and applications of Tilapia (Oreochromis niloticus) fish scale biochars (FSB) as an adsorbent in the removal of indigo carmine dye (ICD) from aqueous solutions is described. The biochars were prepared through pyrolysis over a temperature range of 200 °C–800 °C and characterized for surface charge, functional groups, thermal stability, particle size and morphology, elemental composition, crystallinity, and surface area by using pHpzc, Fourier transform infrared (FTIR) spectroscopy, thermo-gravimetric analysis (TGA), transmission electron microscopy/scanning electron microscopy (TEM/SEM), energy dispersive X-ray (EDX) spectroscopy, powder X-ray diffraction (PXRD) and Brunauer-Emmett-Teller (BET) techniques, respectively. Batch experiments were carried out to determine the variation of adsorption process with initial dye concentration, contact time, initial solution pH, adsorbent load, temperature and adsorbent pyrolysis temperature on the removal of the dye. The percentage removal increased with increase in initial dye concentration and adsorbent dosage. A pH of 2 was the most appropriate for the adsorption experiments. The equilibrium data fitted pseudo-first-order kinetics and Freundlich models, while the thermodynamic parameters confirmed that the adsorption process was endothermic.

Key words | adsorption, fish scale biochar, indigo carmine, kinetics

INTRODUCTION

A number of carbon materials are derived from either plant or animal biomass, which are naturally abundant and renewable materials (Xiu et al. 2017). Important focus has been given to a variety of plant biomass such as energy crops, agricultural remains, and woody biomass (Xie et al. 2015; Okello et al. 2017). Numerous researches have been done on plant biomass (Ngeno et al. 2016) while a very diminutive understanding exists on the feasibility of animal biomass. For example, fish scales are viewed as wastes that do not have a clear economic significance in the environment (Rustad 2003; De Gisi et al. 2016; Nnaemeka et al. 2016; Nia et al. 2017; Temesgen et al. 2018; Fegousse et al. 2019; Lafi et al. 2019), despite their sustainable abundance. However, fish scales have been applied as adsorbents for the removal of copper from water in some countries (Huang 2007). Huang (2007) utilized Tilapia niloticus Linnaeus fish scales instead of Atlantic cod scales, alluding to the fact that tilapia is cheaper and more available compared to Atlantic cod. In addition, it has been shown that applying fish scales as an adsorbent would be a better substitute for removal of metals from waste water compared to wool since fish scales are already a waste generated resource material from fish markets and households (Villanueva-Espinosa et al. 2001; Huang 2007). Rustad (2003) reported that about 91 million tons of fish and shellfish from both inland and marine waters were captured worldwide and 50–60% were consumed by humans and the rest were discarded as waste. Despite the increase in the world’s human population and fish aquaculture, statistics have shown that there is no significant change in fish captured (FAO 2018), and this supports the sustainable availability of fish scales as a resource for production of commercial adsorbents.

Adsorption characteristics of reactive dye onto fish scales in the presence of an electrolyte (NaCl) and surfactant protein (SP) mixtures were studied by Neves et al. (2018) and the results showed favorable kinetics and high adsorption capacity for the RB5G dye, hence providing useful base data for the design, optimization and scaling up of the adsorption processes for commercial application. Bamukyaye & Wanasolo (2017) confirmed that the adsorption of chromium (VI) onto processed fish scales was highly feasible for remediation of effluents from tannery industries. Related work was also reported by Mustafiz (2003) with fish scales from Atlantic cod. Villanueva-Espinosa et al. (2001) examined the suitability of thermally pre-treated fish scales, from the Mojarra Tilapia variety, and observed that the processed adsorbent was effective in the removal of Cu²⁺ ions from wastewater. Effective application of fish scales has also been investigated by other researchers and confirmed to be feasible, and is hence recommended for removal of metal cations and dyes from aqueous solutions (Chowdhury et al. 2012; Ho et al. 2012; Begum & Kabir 2013; Zhu et al. 2013; Abdullah & Vo 2014; Othman et al. 2016; Ooi et al. 2017; Ahmadifar & Koohi 2018; Gholami et al. 2018; Kwaansa-Ansah et al. 2019).

Recently, biochar, a product of biomass thermo-chemical conversion, has received considerable application in the removal of various pollutants owing to its economical and abundance benefits (Xiu et al. 2017). The aim of our study was to employ an abundant local environmental pollutant, fish scales, from a local fish market in Gikomba, Nairobi, Kenya, in the remediation of dye-polluted effluent from some local textile industries. Indigo carmine is a synthetic anionic dye that has been used as an antibacterial agent, biological stain, dermatological agent and additive to poultry feed. It is also a potent carcinogen, which is recallicitrant and toxic to mammalian cells (Owens 2002a, 2002b). The contamination pathway involves conventional treatment plants where the dyes bioaccumulate in sediments and soil and are eventually conveyed to public water supply systems (Vikrant et al. 2018). Fish scale biochars produced in a furnace at 200 °C, 300 °C, 400 °C, 600 °C and 800 °C were prepared following standard procedures and characterized using point of zero charge pH (pHₚzc), X-ray diffraction (XRD) spectroscopy, thermo-gravimetric analysis (TGA), transmission electron microscopy (TEM), Brunauer-Emmett-Teller (BET), scanning electron microscopy (SEM), energy dispersive X-ray (EDX) spectroscopy and Fourier transform infrared (FTIR) spectroscopy in order to understand their physical and chemical properties. Subsequently, preliminary findings for proof of the concept were demonstrated for pulverized raw fish scales and their biochars to rationalize the significance of carrying out the biomass thermo-chemical conversion process. The results of adsorption studies using pulverized raw fish scales and biochars produced at 200 °C, 300 °C, 400 °C, 600 °C, and 800 °C, respectively, are hereby presented.

**EXPERIMENTAL SECTION**

**Materials and instrumentation**

Tilapia (*Oreochromis niloticus*) fish scales were obtained from a local fish market, Gikomba, in Nairobi, Kenya, and extra-pure activated charcoal (99.57%) was purchased from FINAR Limited, Nairobi, Kenya. The chemicals and solvents were of analytical grade and were used as purchased. Indigo carmine dye (ICD) was bought from BDH Chemicals Ltd, Poole, UK. All the solutions for adsorption studies were prepared in deionized water (product 18.2 MΩcm, Ultra 370 Series YOUNGLIN). The instruments: a pH meter (Hanna Instruments Microprocessor pH Meter 211), and an infrared spectrophotometer (Cary 630 FTIR) were used. Surface morphology and particle size of the materials were determined using SEM (Zeiss Evo LS 15 SEM) and TEM (JEOL JEM-1400) respectively. Thermal degradation profile was done on a TGA Thermo plus (Evo2 TG-DTA/H). Crystallinity test was carried out on an XRD, while residual dye concentration was quantified by a UV-Vis spectrophotometer (UVmini-1240-Shimadzu).

**Preparation of fish scales and pulverization**

Fresh Tilapia (*Oreochromis niloticus*) fish scales were first washed with tap water at 25 °C to remove all the dirt, and then rinsed several times with de-ionized water before air drying under shade for 3 days. The dried material was then pulverized at 100 Pa (HERZOG HSM-H). The pulverized fish scales (25.0 g) were placed in a porcelain combustion boat (50 mL), covered with an appropriate lid and subjected to slow pyrolysis in an inert environment (N₂ flow of 100 mL/min) at a heating rate of 10 °C per minute to 200 °C, 300 °C, 400 °C, 600 °C and 800 °C, then for a residence time of 60 minutes using a furnace (WiseTherm). The samples were allowed to cool and were washed with deionized water until the effluents were neutral to litmus (pH 6.7) and finally oven-dried at 100 °C for 2 h (Bordoloi et al. 2018). The adsorbents were stored in
Characterization of the adsorbents

In a typical experiment, 0.3 g was weighed (BAS 31 plus BOECO, Germany) and mixed with 30 mL of deionized water (product 18.2 MΩcm, Ultra 370 Series YOUNGLIN) at 25 °C followed by adjusting of the initial pH (pH_i) of the solutions from 2 to 10 by adding 0.1 M concentration of either HCl or NaOH solutions. The pH_i values were determined by a pH meter (Hanna Instruments Microprocessor pH Meter 211). After a contact time of 5 h, the suspensions were filtered, and the final pH (pH_f) values recorded. The difference between pH_i and pH_f values (ΔpH = pH_f - pH_i) was plotted against pH_i. The pH_pzc of the adsorbent was determined from the point of intersection of the resulting curve, at which pH = 0. The pH values of the adsorbents were measured in deionized water at the ratio of 1:5 wt/wt using a calibrated pH meter (Hanna Instruments Microprocessor pH Meter 211) (Dai et al. 2013).

The functional groups present in each sample were determined on FTIR equipment (Agilent Technologies, Cary 630 FTIR). The changes in surface morphology and elemental compositions were investigated using SEM-EDX analysis (Zeiss Evo LS 15 SEM) at low-pressure vacuum (LVP) with a low vacuum variable pressure secondary electron detector. The particle sizes and crystal structures were obtained using TEM equipment (JEOL JEM-1400). Surface areas, thermal degradation profiles and crystalline phases of the adsorbents were investigated using BET, TGA and XRD.

Screening of the adsorbents and batch experiments

Accurately weighed 0.1 grams of each of the adsorbents of pulverized raw fish scales (RFS) and the fish scale biochars (FSB) were mixed, separately, with 30 mL of prepared standard ICD solutions (at 5, 15, 25, 50, 75 and 100 mg/L) into capped reaction vessels. The vessel components were agitated for 4 h in an orbital shaker/incubator at 200 rpm set at 298 K and the residual dye concentrations were detected using a UV-Vis spectrophotometer (UVmini-1240-Shimadzu) at a wavelength of 611 nm. Batch experiments were performed to probe the effects of solution pH (2–10), initial adsorbate concentration (25–100 mg/L), adsorbate contact time (0–180 min), adsorbent dosage (0.05–0.25 g/30 mL solution) and solution temperature (298–323 K) for ICD on adsorption kinetics using pulverized raw fish scales and biochar materials prepared at different temperatures; FSB@400°C, FSB@600°C, and FSB@800°C. A standard activated charcoal (99.9% purity) was used as a control adsorbent to evaluate the performance of the RFS and the generated biochars. The percentage removal (%) and the amount of dye adsorbed per unit mass of adsorbent were calculated using Equations (2) and (3), respectively.

\[
%R = \frac{C_0 - C_e}{C_0} \times 100 \quad (2)
\]

\[
q_e = \frac{C_0 - C_e}{m} \times V \quad (3)
\]

where \( q_e \), \( C_0 \), \( C_e \), \( m \) and \( V \) are the amount of dye adsorbed (mg/g), initial dye concentration (mg/L), equilibrium dye concentration (mg/L), the mass of the adsorbent (g) and volume of the dye solution (L), respectively. Significant differences in the treatments at 95% confidence (\( P < 0.05 \)) level were obtained using the Statistical Analysis Software (SAS System for Windows Version 8).

Kinetics and mechanisms of adsorptions

In order to investigate the kinetics of adsorption of ICD removal onto the adsorbents, contact time data at different initial dye concentration solutions were fitted into pseudo-first-order, pseudo-second-order, and intraparticle diffusion models represented by Equations (4)–(6), respectively.

\[
\log (q_e - q_t) = \log q_e - \frac{k_1}{2.303} t \quad (4)
\]

\[
\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (5)
\]

\[
q_t = k_p t^{0.5} + I \quad (6)
\]

where \( q_e \) is the equilibrium amount of ICD adsorbed (mg/g), \( q_t \) is the amount of ICD adsorbed at time \( t \) (mg/g), \( k_1 \), \( k_2 \) and \( k_p \) are the adsorption rate constants, respectively, \( I \) is the intercept whose values give an insight on the thickness of the boundary layer and \( t \) is time (min).
Thermodynamic functions; change in Gibb’s free energy ($\Delta G$), enthalpy ($\Delta H$) and entropy ($\Delta S$) were determined using the following equations:

$$\Delta G = -RT \ln K \quad (7)$$
$$K = \frac{C_o - C_e}{C_e} \quad (8)$$
$$\log K = \frac{\Delta S}{2.303R} - \frac{\Delta H}{2.303RT} \quad (9)$$

where $T$ is temperature (Kelvin), $R$ is the universal gas constant ($8.314$ J/mol/K) and $K$ represents the thermodynamic equilibrium constant. Equilibrium adsorption isotherm data were analyzed according to the Langmuir and Freundlich models. The Langmuir model, which is based on the hypotheses that there are uniformly energetic adsorption sites, monolayer coverage, and no lateral interaction between adsorbed molecules, is suitable in describing the adsorptive behavior of homogeneous surfaces. The mathematical representation of the non-linear Langmuir adsorption model is given below:

$$q_e = \frac{q_m K L C_e}{1 + K L C_e} \quad (10)$$

where $K_L$ is the Langmuir equilibrium constant (L/mg) and $q_m$ the maximum adsorption capacity of the adsorbent (mg/g). Non-linear regression of equilibrium adsorption data for each isotherm was performed by minimizing the regression sum of squares (RSS) error function given by:

$$RSS = \sum_{i=1}^{N} (q_{e,\text{predicted}} - q_{e,\text{experimental}})^2 \quad (11)$$

where $N$ is the number of data points.

RESULTS AND DISCUSSION

Synthesis and characterization of fish scale biochars

The fish scale biochars were prepared via slow pyrolysis at different temperatures of $200^\circ C$ (FSB@200°C), $300^\circ C$ (FSB@300°C), $400^\circ C$ (FSB@400°C), $600^\circ C$ (FSB@600°C) and $800^\circ C$ (FSB@800°C). The percentage yields of each FSB prepared at a specified temperature are given in Table 1. From the results, it was observed that biochar yields decreased significantly with increase in pyrolysis temperatures, an observation attributed to liberation of volatile matter such as water, carbon dioxide, ammonia, carbon monoxide, $C_xH_yO_z$ and hydrogen cyanide during pyrolysis (Xu & Chen 2013). Xu & Chen (2013) also reported that the mineral component in the biochar functions as a barrier to the diffusion of heat, causing the release of the volatile components during the charring process, an observation that is in agreement with several other previous reports (Uchimiya et al. 2011; Jindo et al. 2014; Liu et al. 2015; Li et al. 2016; Bordoloi et al. 2018).

### Determination of point of zero charge pH (pHpzc) and pH of the adsorbents

Point of zero charge (pHpzc) of a material predicts the net charge on the surface of an adsorbent at a given solution pH. Thus, the adsorbent surface becomes positively charged when the solution pH is less than the pHpzc. Therefore, high values of pHpzc promote adsorption of anionic adsorbates. Conversely, an adsorbent bears a negative surface charge and effectively adsorbs cations if the solution pH values are greater than the pHpzc (Gholami et al. 2018). The values for the point of zero charge pH (pHpzc) are highlighted in Table 1, while the plots are given in Figure S1 (Supplementary Information). In this study, the pHpzc values of the adsorbents ranged from 6.48 to 8.42. Uchimiya et al. (2011) recorded values of 3.5–10.1 for biochar adsorbents derived from cottonseed hulls prepared at temperatures of between 200°C and 800°C. Our pHpzc values indicate that the surface of the adsorbents would acquire either positive or negative charges in an adsorbate solution depending on the solution pH. Similar findings were reported for the activated surface of banana and orange peels adsorbents (Temesgen et al. 2018). In this study, the pH(H2O) levels registered by the biochars increased with pyrolysis temperature. Rafiq et al. (2016)

### Table 1 | Percent (%) yield of biochars

<table>
<thead>
<tr>
<th>Adsorbent</th>
<th>% Yield</th>
<th>pHpzc</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw fish scales</td>
<td>–</td>
<td>8.42</td>
<td>7.65</td>
</tr>
<tr>
<td>Activated charcoal</td>
<td>–</td>
<td>3.80</td>
<td>7.37</td>
</tr>
<tr>
<td>FSB@200°C</td>
<td>77</td>
<td>7.67</td>
<td>6.29</td>
</tr>
<tr>
<td>FSB@300°C</td>
<td>57</td>
<td>7.36</td>
<td>7.25</td>
</tr>
<tr>
<td>FSB@400°C</td>
<td>53</td>
<td>7.27</td>
<td>7.05</td>
</tr>
<tr>
<td>FSB@600°C</td>
<td>51</td>
<td>6.97</td>
<td>7.95</td>
</tr>
<tr>
<td>FSB@800°C</td>
<td>46</td>
<td>6.48</td>
<td>8.05</td>
</tr>
</tbody>
</table>

Note: pHpzc: point of zero charge pH.
also reported a decrease in %H contents of the biochars as pH and pyrolysis temperature increased.

**Fourier transform infrared spectroscopy**

The FTIR spectra of the FSB are given in Figure 1. These adsorbents were majorly composed of C, O, Ca and P. The bands at 3,414 cm$^{-1}$ and 3,084 cm$^{-1}$, whose intensity decreased with increasing pyrolysis temperature, correspond to -OH stretching vibrations attributed to the alcoholic functional group (Zainon et al. 2012; Slimani et al. 2014; Paul et al. 2017) while those at 1,632 cm$^{-1}$ and 1,581 cm$^{-1}$ characterized the amides (Zainon et al. 2012; Paul et al. 2017). Carbonate group stretching frequencies were assigned to the bands at 1,456 cm$^{-1}$ and 1,440 cm$^{-1}$ while those at 1,021 cm$^{-1}$ and 1,014 cm$^{-1}$ correspond to C-O stretching vibrations; peak of PO$_4^{3-}$ groups of hydroxyapatite was assigned at wavenumber in the range of 1,040–1,010 cm$^{-1}$ (Zainon et al. 2012; Slimani et al. 2014; Paul et al. 2017). Absorption band of the carboxylate group on the surface of activated charcoal was evidenced by the band at 1,650 cm$^{-1}$ (Bagtash & Zolgharnein 2012).

**Surface morphology, structure and chemical composition**

The morphology, structure, particle size and surface area of the FSB adsorbents were also studied using SEM-EDX, TEM, BET and powder X-ray diffraction (PXRD). The SEM micrographs showed that the charred fish scales appeared more compact compared to the pulverized raw fish scales, a consequence of thermo-chemical conversion (Figure 2(a) and 2(d) and Figure S2). This is confirmed by difference in the image of the activated charcoal (Figure 2(c)), which is also as a result of thermo-chemical conversion. The TEM micrograph of the FSB@400°C (Figure 2(d)), FSB@600°C and FSB@800°C (Figure S3) adsorbents displayed spotted ring-like patterns with particle sizes of 24.21 nm, 17.43 nm and 22.92 nm, respectively.

The EDX results showing the elemental composition of the adsorbents are given in Table 2. Activated carbon had the highest carbon content of 84.40% followed by FSB@600°C, FSB@800°C, FSB@400°C and raw fish scales. High carbon contents observed in the biochars were attributed to inorganic carbon as a result of pyrolysis. The percentage of other inorganic elements such as Ca, Na and Mg also increased with pyrolysis temperature while N, O and P exhibited unpredictable trends. This observation is in agreement with the findings of Rafiq et al. (2016). There were negligible deviations of Na, Mg, Ca, P and O levels in FSB@400°C and FSB@400°C-IC. However, concentrations of N, C, H and S significantly increased in FSB@400°C-IC, which confirmed the adsorption of ICD.

The mineral composition and phase purity of the fish scale biochars were examined by XRD and the diffraction patterns are presented in Figure 3 and Figure S4. The fish scale biochars (FSB@400°C, FSB@600°C, and FSB@800°C) are mainly composed of hydroxyl apatite (Ca$_5$(PO$_4$)$_3$(OH)) phase (Zainon et al. 2012; El Haddad et al. 2013a; Slimani et al. 2014; Scapin et al. 2015; Paul et al. 2017). Such results were also reported by Othman et al. (2016). The results showed that fish scale biochar at 400°C (FSB@400°C) exhibits broader peaks compared to FSB@600°C and FSB@800°C, whose peaks are sharper and more intense. This difference may be attributed to the presence of proteins and less complete decomposition of organic matter at 400°C (Paul et al. 2017).

Surface area and pore volume are some of the most significant adsorptive properties of an adsorbent. The surface areas and pore volumes of the adsorbents were determined and the results were as shown in Table 3. The results
displayed an increase in both surface area and pore volume as the pyrolysis temperature increased up to 600 °C, thereafter decreasing at 800 °C. Precisely, raw fish scales and activated charcoal recorded the least and the highest surface area and pore volume, respectively. This finding clearly suggests that, in general, pyrolysis improves the surface area of a biomass adsorbent (Rafiq et al. 2016).

**Thermo-gravimetric analysis (TGA)**

The thermal properties and stability of the adsorbents were investigated using thermo-gravimetric analysis (Figure 4). The recorded initial weight loss above 100 °C is due to loss of moisture from the fish scales, while the subsequent sharp drop in weight between 200 °C and 700 °C could be ascribed to the elimination of organic matter and dehydration of HPO$_4^{2-}$ yielding hydroxyl apatite (HAp) (Ca$_5$(PO$_4$)$_3$(OH)) (Zainon et al. 2012; Paul et al. 2017). In addition, there was a minor drop in weight at 780 °C, owing to the elimination of glycine and other organic proteins from the fish scale, beyond which there was no noticeable significant change (Zainon et al. 2012; Paul et al. 2017). For the biochars (Figure 4(b)), thermal degradation profiles revealed that percent weight loss was

<table>
<thead>
<tr>
<th>Adsorbents</th>
<th>C%</th>
<th>N%</th>
<th>H%</th>
<th>O%</th>
<th>Ca%</th>
<th>P%</th>
<th>Mg%</th>
<th>Na%</th>
<th>S%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw fish scales</td>
<td>6.8</td>
<td>–</td>
<td>–</td>
<td>37.74</td>
<td>15.20</td>
<td>8.97</td>
<td>0.34</td>
<td>0.16</td>
<td>0.08</td>
</tr>
<tr>
<td>Activated charcoal</td>
<td>84.40</td>
<td>8.26</td>
<td>–</td>
<td>6.78</td>
<td>0.26</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>FSB@400°C</td>
<td>12.51</td>
<td>4.07</td>
<td>1.07</td>
<td>40.51</td>
<td>27.38</td>
<td>12.98</td>
<td>0.39</td>
<td>0.34</td>
<td>–</td>
</tr>
<tr>
<td>FSB@600°C</td>
<td>18.41</td>
<td>0.37</td>
<td>0.06</td>
<td>43.74</td>
<td>29.08</td>
<td>13.89</td>
<td>0.46</td>
<td>0.32</td>
<td>–</td>
</tr>
<tr>
<td>FSB@800°C</td>
<td>14.72</td>
<td>0.50</td>
<td>0.01</td>
<td>37.04</td>
<td>32.28</td>
<td>14.90</td>
<td>0.42</td>
<td>0.20</td>
<td>–</td>
</tr>
<tr>
<td>FSB@400°C-IC</td>
<td>35.50</td>
<td>16.74</td>
<td>1.14</td>
<td>40.48</td>
<td>27.34</td>
<td>12.92</td>
<td>0.38</td>
<td>0.28</td>
<td>0.06</td>
</tr>
</tbody>
</table>
inversely proportional to the temperature of pyrolysis. Weight reduction during thermal analysis did not exceed 10% for both FSB@600°C and FSB@800°C; however, approximately 30% decrease in weight was recorded for FSB@400°C. This trend was also observed by Jindo et al. (2014) for biochars derived from different agricultural residues.

### Screening of the raw fish scale, fish scale biochars and activated charcoal in removal of ICD

The pulverized raw fish scales and fish scale biochars were investigated as adsorbents in the removal of ICD from aqueous solution using adsorbent dosage of 0.1 g/L, solution volume of 30 mL, pH of 8.5 at room temperature. The results for all the adsorbents are summarized in Table 4.

The adsorbents recorded significantly different ($p \leq 0.05$) percent levels of dye removal (%R); with pulverized raw fish scales (RFS) and the biochar (FSB@600°C) performing the least (values between 13.68 at 15 mg/L and 24.14 at 100 mg/L) and the most effectively (values between 39.56 at 15 mg/L and 59.81 at 100 mg/L) respectively. The trend of the mean performance of the adsorbents was RFS < FSB@400°C < FSB@800°C < FSB@600°C < activated charcoal (18.09, 22.88, 27.06, 44.07, 48.27 and 90.88%). The trend reveal that FSB performed better than

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**Table 3 | Surface areas and pore volumes of the adsorbents**

<table>
<thead>
<tr>
<th>Adsorbent</th>
<th>Surface area (micropore surface area), m² g⁻¹</th>
<th>Pore volume (micropore volume), cm³ g⁻¹</th>
<th>Particle size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw fish scales</td>
<td>2.8</td>
<td>0.002</td>
<td>444.13</td>
</tr>
<tr>
<td>Activated charcoal</td>
<td>1.024</td>
<td>0.71</td>
<td>$5.8 \times 10^{-8}$</td>
</tr>
<tr>
<td>FSB@400°C</td>
<td>40.79</td>
<td>0.11</td>
<td>22.92</td>
</tr>
<tr>
<td>FSB@600°C</td>
<td>94.05</td>
<td>0.23</td>
<td>17.45</td>
</tr>
<tr>
<td>FSB@800°C</td>
<td>47.77</td>
<td>0.20</td>
<td>24.21</td>
</tr>
</tbody>
</table>

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**Figure 3 | Powder X-ray diffractograms of biochars prepared at 400 °C, 600 °C and 800 °C.**

**Figure 4 | TGA thermograms of (a) raw fish scales and (b) fish scale biochars.**
Batch adsorption process and conditions

Batch adsorption experiments were carried out to determine the influence of contact time, initial dye concentration, adsorbent dosage, initial solution pH and temperature on the adsorption of ICD. The effect of contact time and initial dye concentration on adsorption were studied using 25 mg/L, 50 mg/L, 75 mg/L and 100 mg/L of dye solutions at 298 K with FSB@600°C dosage of 0.20 g/30 mL, 200 rpm from 0 to 180 minutes. Figure 5 shows plots of percentage dye removal over a period of time at different initial dye concentrations. From Figure 5(a), the adsorption process was very rapid during the initial stage, followed by a gradual phase as the system attained equilibrium. The equilibrium time varied with the initial dye concentration. For instance, dosages of 25 mg/L, 50 mg/L, 75 mg/L and 100 mg/L attained maximum adsorptions after 40 min, 10 min, 40 min and 50 min, respectively. In addition, more dye molecules were adsorbed at higher concentrations than at lower ones. For example, adsorption percentages of 48.59%, 52.95%, 62.54% and 72.47% were recorded for dye dosages of 25 mg/L, 50 mg/L, 75 mg/L and 100 mg/L, respectively. The fast initial adsorption rate is attributed to a large number of vacant adsorption sites available for occupancy by the adsorbate molecules (Slimani et al. 2014; Adeyi et al. 2019). The slow adsorption towards equilibrium point may be assigned to repulsive forces between the dye molecules in the bulk solution and the adsorbed molecules (Shikuku et al. 2018) and the reduced number of available binding sites. Notably, more dye was adsorbed on untreated fish scales was attributed to their non-porous surfaces (Kabir et al. 2019).

### Table 4 | Percent dye removal (%R) by raw fish scales, fish scale biochars and activated charcoal

<table>
<thead>
<tr>
<th>Adsorbents</th>
<th>ICD concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 mg/L</td>
</tr>
<tr>
<td>Raw fish scales</td>
<td>13.90f</td>
</tr>
<tr>
<td>FSB@200°C</td>
<td>20.41e</td>
</tr>
<tr>
<td>FSB@300°C</td>
<td>25.85d</td>
</tr>
<tr>
<td>FSB@400°C</td>
<td>34.53c</td>
</tr>
<tr>
<td>FSB@600°C</td>
<td>39.56b</td>
</tr>
<tr>
<td>FSB@800°C</td>
<td>35.52c</td>
</tr>
<tr>
<td>Activated charcoal</td>
<td>82.73a</td>
</tr>
</tbody>
</table>

| Mean concentration | 36.07f  | 37.92e  | 39.77d  | 42.42c  | 44.08b  | 51.85a  | 42.02         |
| CV%               | 6.13    | 3.35    | 2.88    | 2.95    | 0.45    | 0.37    | 2.99          |
| LSD, p ≤ 0.05     | 3.87    | 2.22    | 2.00    | 2.20    | 0.34    | 0.33    | 0.83          |

Adsorbent dosage 0.1 g/L, solution volume 30 mL, solution pH 8.5, ambient temperature, contact time of 4 h; a, b, c, d, e, f, g signify significant differences; LSD, least significant differences; CV, coefficient of variation.

pulverized RFS, respectively. The trend is an indicator of the surface characteristics of the biochar that are different from those of the pulverized RFS and are influenced by the pyrolysis temperature. The observed consistent disparity in performance between RFS and the biochars underscores the necessity of thermal conversion of the biomass, which improved adsorbent surface characteristics such as surface area and pore volume that are important for the adsorption process (Rafiq et al. 2016). From the data in Table 4, surface area and particle size exhibited an inverse relationship. For example, as the particle size decreased from 22.92 nm in FSB@400°C to 17.43 nm in FSB@600°C, the removal efficiency increased from 44.07 to 48.27%. The increase in removal efficiency with decreasing particle size is consistent with increase in the effective surface area (40.79 and 94.05 m²/g) of adsorbents in the same order. Mashkoor et al. (2018) reported a decrease in adsorption of chromium (VI) on egg-shell and fish-scale powder with the increase in particle size for all initial adsorbate concentrations. The poor adsorption witnessed on untreated fish scales was attributed to their non-porous surfaces (Kabir et al. 2019).
The influence of adsorbent dosage on the dye removal efficiency was studied by varying the dosage from 0.05 g/30 mL to 0.25 g/30 mL as given in Figure 5(b). The results indicate that the removal efficiency (%R) increased with an increase in the amount of adsorbent. As an illustration for FSB@600/C14, the removal efficiency (%R) significantly (p < 0.05) increased from 52.70% at an adsorbent dose of 0.05 g to 74.40% at 0.20 g. This was expected due to the increase in the number of active sites (Ngeno et al. 2016; Nnaemeka et al. 2016; Bordoloi et al. 2018; Temesgen et al. 2018; Adeyi et al. 2019; Badeenezhad et al. 2019; Fegousse et al. 2019; Tsiele et al. 2019). Optimum adsorbent dosages were recorded as 0.10 g, 0.25 g, 0.20 g and 0.20 g per 30 mL, respectively, for activated charcoal, FSB@400/C14, FSB@600/C14, and FSB@800/C14, respectively. Gholami et al. (2018) observed an optimum dosage of fish scale composites at 0.30 g in the removal of methyl blue dye. Decrease in dye removal beyond the optimum dosages could be due to adsorbent aggregation leading to decreased total surface area (Bamukyaye & Wanasolo 2017; Gholami et al. 2018; Neves et al. 2018; Ayanda et al. 2019).

Next, we examined the influence of pH on dye adsorption within the pH range of 2–10. Previous studies have established that adsorbent performance at various pH levels is controlled by surface charges, active sites and adsorption capacity of the adsorbent (Santhi et al. 2016). In the current study, percent removal (%R) of ICD from aqueous solution was observed to decrease gradually with increase in solution pH for all the adsorbents (Figure 5(c)). Adsorbent FSB@600°C recorded a decrease in removal efficiency from 56.60% at pH 2 to 49.55% at pH 10. This can be accounted from the fact that at pH below 6, the adsorbents acquire net positive surface charges as predicted by the point of zero charge pH (pHpzc) (Table 1 and Figure 6(e)). Attractive coulombic forces occurred between the adsorbent and anionic ICD molecules (Figure 6(e)), hence the high adsorption capacity (Nnaemeka et al. 2016; Ayanda et al. 2019; Badeenezhad et al. 2019). On the other hand, at higher pH levels, the hydroxyl (OH−) ions in the aqueous solution compete effectively with the dye anions, causing a decrease in the percent removal (Slimani et al. 2014). In a previous study by Chakraborty et al. (2016), a higher pH of 8 resulted in lower removal efficiencies of methyl orange (MO) dye molecules. It is noteworthy to state that the adsorption occurred quite uniformly within a wide range of pH, demonstrating the potential of these adsorbents for application in real environmental remediation for a variety of industrial effluents (Bordoloi et al. 2018). The optimum pH 2 for dye removal in the present work is in agreement with the finding reported by Almoisheer et al. (2019).

In order to investigate the effect of temperature on the adsorption of ICD, the adsorption experiments were carried out at different temperatures ranging from 290 K to 330 K (Kelvin) (Figure 5(d)).
out at 298 K, 303 K, 313 K and 323 K at different initial dye concentrations and at an initial solution pH 2, adsorbent dosage of 0.20 g/30 mL solution (Figure 5(d) and Figures S5a and S6a). From these figures, it was evident that the amount of dye removed increased with increase in temperature for all the adsorbents, with an exception for activated charcoal at 323 K. For example, dye removal efficiency of FSB@600°C significantly (p < 0.05) increased from 45.69% at 298 K to 72.04% at 323 K. These findings suggest the endothermic nature of these adsorption processes (Kulkarni et al. 2011). Ho & McKay (2003) also reported that higher temperatures enhanced adsorption due to the increased kinetic energy of the adsorbate and adsorbent surface activity.

The kinetics and thermodynamics of adsorption

In order to investigate the kinetics of adsorption of ICD removal onto the adsorbents, the contact time data at various initial adsorbate concentrations were fitted onto pseudo-first-order, pseudo-second-order, and intraparticle diffusion models and are presented in Table 5. The $R^2$ values for ICD concentrations of 25, 50, 75 and 100 mg/L were 0.987, 0.611, 0.801 and 0.947, respectively, signifying that $q_e$ had a low linear dependency on $t^{0.5}$ (Figure S6d). From the data in Table 5, it can be concluded that intraparticle diffusion ($K_p$) was not the sole operating rate controlling step, since the plot registered non-zero y-intercept.

### Table 5 | Calculated parameters for kinetic models

<table>
<thead>
<tr>
<th>Conc. (mg/L)</th>
<th>Pseudo-first order</th>
<th>Pseudo-second order</th>
<th>Intraparticle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_1$ (1/min)</td>
<td>$q_e$ (mg/g)</td>
<td>$R^2$</td>
</tr>
<tr>
<td>25</td>
<td>58,535</td>
<td>1.778</td>
<td>0.99999</td>
</tr>
<tr>
<td>50</td>
<td>58,537</td>
<td>4.040</td>
<td>0.99999</td>
</tr>
<tr>
<td>75</td>
<td>58,537</td>
<td>6.950</td>
<td>0.99999</td>
</tr>
<tr>
<td>100</td>
<td>58,532</td>
<td>10.689</td>
<td>0.99999</td>
</tr>
</tbody>
</table>
values \((C > 0)\) (El Haddad et al. 2013b; Okello et al. 2017). Comparison of \(R^2\) values showed that equilibrium data best fitted the pseudo-second-order kinetic model since they are all close to unity.

Thermodynamic parameters; \(\Delta G\), \(\Delta H\), and \(\Delta S\) were also calculated (Table S1 and Figures S5b, S6b and S6c). Positive \(\Delta H\) values indicated that the adsorption processes were endothermic, corroborating the observed increase in adsorption with temperature. Similarly, the positive \(\Delta S\) denoted an increase in randomness at the solid/liquid interface. Adsorption onto the activated charcoal was spontaneous at all temperatures as seen in the negative \(\Delta G\) values. However, adsorptions onto the fish scales chars were predominantly non-spontaneous and this non-spontaneity decreased with temperature. It is noteworthy that the spontaneity also varied with the initial concentration, denoting its dependence on the equilibrium constant \(K\). From the data, it can be deduced that chemisorption was the principal mechanism of ICD removal from the aqueous solution onto the adsorbents (Kulkarni et al. 2017). Begum & Kabir (2013) reported that the functional groups in the fish scale structure, such as phosphates, carboxyls, amines and carbonyls, are supposed to be involved in the sorption process as confirmed by the FTIR spectra in this study.

**Adsorption isotherm modeling**

Isotherm models are fundamental concepts of the adsorption phenomenon that explain the interaction between the adsorbate and adsorbent (Nia et al. 2017). The non-linear Langmuir and Freundlich forms were used to fit the adsorption data (Table S2). The constants \(q_m\), \(K_L\) and \(n\) are the Langmuir maximum monolayer adsorption capacity (mg/g), the Langmuir constant representing the energy of adsorption (L/mg), the Freundlich constant (mg/g. Lmg\(^{-1}\cdot\)L\(^{-1}\)) and the Freundlich model constant, which indicates the intensity of the adsorption process. The Freundlich models displayed better fit for the data than the Langmuir isotherm, based on \(R^2\) values, suggesting multilayer adsorption. The magnitude of \(n\) describes the favorability of the adsorption process. The \(n\) values ranging 2–10 represent good, 1–2 for moderately difficult, and less than 1 for a poor adsorptive potential (Treybal 1980). In the present study, the magnitudes of \(n\) were averagely <1, suggesting a poor adsorptive potential.

**Characterization of the spent adsorbents FSB**

In attempts to establish any morphological and chemical changes to the adsorbents during adsorption, we analyzed the used adsorbents using FTIR and SEM-EDX (Figure 6). Notably, the SEM image of fresh FSB@400°C exhibited morphologically different surfaces relative to the used FSB@400°C-IC (Figure 6(a)). This could be due to aggregation of adsorbate molecules on the adsorbent surface (Bordoloi et al. 2018). In addition, elemental composition analysis by EDX revealed the presence of N, H, S and Na (Table 3 and Figure S6e), supporting the adsorption of ICD on FSB@400°C. Gholami et al. (2018) reported the presence of N and S elements from methyl blue (MB) dye on the surface of used magnetic fish scales (UMFS), confirming the adsorption of the dye. In addition, the spots of FSB@400°C-IC on the TEM micrograph were blurred due to surface coverage with dye molecules (Figure 6(b)). The FTIR spectrum of the used adsorbent (Figure 6(d)) displayed some changes on the surface of the adsorbent. For instance, the peaks at 3,008, 2,374, 2,151, 1,489 and 587 cm\(^{-1}\) shifted to 3,006, 2,385, 2,134, 1,454 and 562 cm\(^{-1}\). Similarly, the observed reductions in the intensities of some peaks, demonstrates the interaction between the dye and the adsorbents (Ooi et al. 2017).

**CONCLUSIONS**

Pulverized raw fish scale and fish scale biochars were successfully prepared and characterized for surface charge, functional groups, thermal stability, particle size and morphology, elemental composition, crystallinity, and surface area by using pHpzc, FTIR, TGA, TEM/SEM, EDX, powder XRD and BET techniques, respectively, and applied as adsorbents for the removal of ICD from aqueous solutions. The biochars performed significantly better than raw fish scales in ICD removal. The percent (%) removal on the surface of the biochar increased with increase in adsorbent dosage and initial dye concentration. The peak adsorbent dosages were 0.10 g, 0.25 g, 0.20 g, and 0.20 g for activated charcoal, FSB@400°C, FSB@600°C, and FSB@800°C, respectively. pH 2 was the most favorable for the adsorption of ICD. The data fitted the pseudo-first-order kinetic model while the Freundlich model displayed a better fitting than the Langmuir isotherm, signifying multilayer adsorption. Adsorptions onto the fish scale biochars were predominantly non-spontaneous and the non-spontaneity decreased with temperature and varied with initial concentration, denoting its dependence on the equilibrium constant \(K\). The magnitudes of favorability of adsorption process, \(n\), were <1, indicating poor adsorptive potential.
CONFLICTS OF INTEREST

The authors declare that there is no conflict with any other third party.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this paper is available online at https://dx.doi.org/10.2166/wst.2020.040.

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First received 18 November 2019; accepted in revised form 21 January 2020. Available online 4 February 2020