Utilization of *Chlorella vulgaris* after the Extraction Process in Wastewater Treatment as a Biosorption Material for Ciprofloxacin Removal

Enass S.M. Al-Mashhadani[1*], Mahmood K.H. Al-Mashhadani[1]

[1] Department of Chemical Engineering, College of Engineering University of Baghdad,Iraq
* Corresponding author's e-mail: enass.mageed1607m@coeng.uobaghdad.edu.iq

ABSTRACT

Potentially negative environmental and human health effects have led to pharmaceutical chemicals,which are labeled as a new class of environmental contaminants. Adsorption is one of the most appealing choices to remove that pharmaceutical waste in recent years. However, the environmental limitations of the adsorbent material are an obstacle to the development of this process. The current study suggested the remaining *Chlorella vulgaris* biomass, after the extraction process of the biomaterials, to be a bio-absorption material in removing the Ciprofloxacin from the hospital wastewater. The preparation and characterization of the suggested adsorbent through FTIR analysis, and scanning electron microscopy, coupled with energy-dispersive X-ray spectroscopy and X-ray diffraction, were present in the current study. Several functional groups (such as carboxylic, amines, hydroxyls, and amides) were observed to aid the adsorption process. After the extraction process, the results showed a growth in the peaks, indicating an increase in functional groups, particularly the -O.H. and -N.H. groups, while having changed toward lower energy after binding with CIP atoms, as well as an increase in surface area from 2.3723 to 3.6224 m²/g. The XRD was shown to be compatible with the EDX test, which both demonstrated a decrease in carbon element concentration due to the deconstruction process. The effects of Ciprofloxacin bio-sorption variables, including contact time, initial Ciprofloxacin concentration, pH, and adsorbent dosage, were adopted as a parametric study. The maximum adsorption capacity was recorded at pH 7 with an adsorbent dose of 2.75 g/L; after 120 minutes, the data show that 89.9% of Ciprofloxacin has been adsorption onto the extracted biomass.

Keywords: microalgae; *Chlorella vulgaris* biomass; adsorption; wastewater treatment; ciprofloxacin.

INTRODUCTION

Pharmaceutical compounds are new contaminants that could negatively impact the environment and human health. Due to medicines' inability to biodegrade in aquatic environments, even at low levels, water contamination with these substances is seen as a growing hazard (Klavarioti et al., 2009). Through biomagnification, harmful quantities of pharmaceutical compounds can build up in food webs, negatively affecting high-trophic creatures like people, especially pregnant women, young children, and the elderly with weaker immune systems (Kelly et al., 2007). The investigations highlight both the ineffectiveness of many conventional wastewater treatment plants and the ongoing entry of these chemicals and their metabolites into sewage waterways (Mohammed-Ridha et al., 2014). Pharmaceuticals are primarily released into the environment through the discharge of treated effluents from conventional wastewater treatment plants WWTPs, via domestic and hospital wastewaters, either directly or by leaching the sludge produced by these facilities or through discharges from the pharmaceutical industry (Möller et al., 2000; Buerge et al., 2003; Deziel, 2014). Hospital wastewater poses risks to the environment in the form of physical waste, chemicals, and biological hazards; pharmaceutical compounds pose the greatest chemical risk and are often discharged to sewage treatment plants without first undergoing

primary treatment. These facilities are unsuitable for treating pharmaceutical compounds since they are designed to remove more general pollutants such as oils, fats, organic matter, nitrogen, and phosphorus, which results in the leaking of these substances into the environment (Zhang et al., 2020). They are regularly discovered in groundwater, surface water, and wastewater effluent (Lindsey et al., 2001; Gimeno et al., 2016). Antibiotics and their breakdown products are frequently found in the environment due to the widespread use of antibiotics to treat microbial infections, the prevention of disease, and stimulation of plant and animal growth (Hirsch et al. 1999, Kümmerer 2009). Most commonly used in developing nations, antibiotics such as penicillins, sulphonamides, cephalosporins, macrolides, and quinolones had a 36 percent increase between 2000 and 2010 (Thung et al., 2016). Several synthetic antimicrobial compounds known as fluoroquinolones have a wide range of antibacterial activity against organisms (Abbas et al., 2016). Fluoroquinolone antibiotics (F.Q.s) and their transformation byproducts represent an important family of E.C.s. The fundamental issue with F.Q.s is the possibility of bacterial resistance genes emerging and the ramifications for human health. As an illustration, due to repeated exposure to hospital wastewater, Acinetobacter baumannii was discovered to be immune to all available antibiotics (Dijkshoorn et al., 2007). Ciprofloxacin, a fluoroquinolone antibiotic, was chosen as the example drug since it is often administered (Picó et al., 2007). This antibiotic, commonly abbreviated as CIP, has been shown to be effective in treating a wide range of diseases in humans and animals (Karthikeyan, 2005; Aristilde et al., 2010). Ciprofloxacin is a synthetic chemotherapeutic agent. It has a carboxylic group with a pKa1 value of 6.1 and an amine group with a pKa2 value of 8.7 in the piperazine moiety. Its molecular weight is 331.4 g/mol (Githinji et al., 2011). The third-generation fluoroquinolone ciprofloxacin fights both gram-positive and gram-negative bacteria by inhibiting DNA unwinding and replication in bacteria. Due to the inefficiency of conventional water treatment methods in removing CIP from water, it has been widely detected in a wide variety of water sources, including surface water, groundwater, wastewater, and raw milk (Hirsch et al., 1999; Kolpin et al., 2002; Kümmerer, 2009; Lapworth et al., 2012; Han et al., 2015). For soil and effluent systems at varying pH levels, Ciprofloxacin has excellent solubility in aqueous mediums (Jiang et al., 2013). Antibiotics from water resources can be removed using a variety of techniques. These processes include some that occur after more typical biological processes, like membrane processes (Acero et al., 2010), ion exchange (Wang et al., 2016), and reverse osmosis (Rodriguez-Mozaz et al., 2015). Activated carbon (A.C.) is a perfect adsorbent since it can remove various dangerous contaminants. The most significant drawbacks of A.C. are its high cost and The inability to generate or recycle (Gupta et al., 2009; Marchal et al., 2013; Yadav et al., 2013). Enhanced oxidation processes (AOPs) are regarded as a competitive water treatment technology for breaking certain organic micropollutants that cannot be eliminated by biological (De la Cruz et al., 2012; Audenaert et al., 2013). However, AOPs' high operating and upkeep expenses prevent them from being used in extensive applications (Ramanan et al., 2016). The sorption method is preferred over other methods due to its worldwide application, design simplicity, and operation convenience (Gupta et al., 2013). Adsorption is a separation process involving the accumulation of a liquid or gaseous adsorbate at the surface and the inter pores of a solid adsorbent. This process is acknowledged as a highly efficient, low-cost, and environmentally friendly wastewater treatment technique. The type of adsorbent and the adsorption parameters have the most effects on adsorption efficiency (Ahmed et al., 2020). Therefore, cost-effective adsorbents were used to remove various contaminants, including agricultural wastes, clay materials, biomass, inert algae, and waste from preparing seafood (Irem et al., 2013). Numerous biosorbents' capabilities were discovered to be extremely high compared to other adsorbents, Natural zeolites, activated carbon, and synthetic ions. Because these types of biosorbents' cell walls comprise polysaccharides and proteins with different functional groups, such as amine, carboxyl, hydroxyl, sulfates, and phosphates, to blame for interaction with contaminants (Sulaymon et al., 2012). Microalgae is an environmentally friendly and sustainable feedstock for producing a wide range of high-value bioproducts and biofuels (Li et al., 2008). Both living and dead microalgae are increasingly being employed to remove antibiotics and other hazardous chemical pollutants from the environment (Santaeufemia et al., 2016; Xiong et al., 2016). Extraction of substances

(such as lipids and fatty acids) from algal biomass utilizing a solvent. Even though marine green algae have a low extractable oil concentration, it is possible to make biodiesel from them using common organic solvents (Xie et al., 2014). Waste biomass from microalgae biodiesel production could harm the environment if not properly disposed of (Zheng et al., 2012). Therefore the use of the leftover microalgal biomass as an adsorbent after the lipid content is extracted might also represent a means to improve the economic viability of the manufacture of an adsorbent from microalgae, in addition to their high availability, cheap cost, and adsorption capacity (Mata et al., 2010). Antibiotic adsorption using dead microalgae biomass is extremely reliant on the antibiotic and microalgae used due to hydrophobicity, functionality, and structure changes. It is best if the antibiotic is hydrophobic than hydrophilic and has a charge opposite to microalgae (Leng et al. 2015; Xiong 2019). Among the many methods of treating wastewater, biosorption is becoming increasingly popular. Toxic substances are taken up passively by dead microalgal biomass. Even though most biosorption studies in the literature address heavy metal removal (Suresh Kumar et al., 2015). The current study examines the potential application of a new class of inexpensive materials using leftover C. vulgaris biomass, but after extraction, asa biosorbent for the elimination ofpharmaceutical compounds from the local hospitals. The ciprofloxacin compound was chosen based on research conducted on sewage water coming out of the unitedhospitals in Baghdad (Al-Imameen and Al-Yarmouk).

MATERIAL AND METHODS

Analyses of the actual sewage

Sewage samples from the Al-Imameen and Al-Yarmouk hospitals were taken and analyzed using chromatography HPLC model SYKAMN in the research center and food pollution/environment and water research at the Ministry of Science and Technology. The analyses revealed the presence of three types of antibiotics with high rates, namely: Ciprofloxacin, levofloxacin, and tetracycline; Ciprofloxacin was selected to verify its removal. According to these preliminary results, Ciprofloxacin (CIP) stock solutions were made by dissolving the needed amount of antibiotics in distilled water and then diluting them to

the desired concentrations. Preparations for both NaOH (0.1 M) and HCl (0.1 M) solutions were made to achieve the selected pH range for the antibiotic solution.

Chemicals and reagents

The antibiotic Ciprofloxacin $C_{17}H_{18}FN_3O_3$, as shown in Figure 1 (purity >98%, molecular weight M.W 331.3 g/mol), was supplied by local AL KINDI pharmaceutical industries Company. Other chemicals were ethanol (C2H60, purity 99.7%, M.W. 46.07) supplied by (Alpha Chemika. INDIA) and chloroform (CHCL3, purity 99.0%, M.W. 119.38) from (THOMAS BAKER.INDIA).

Post-preparation of biosorbent

dried green cells of *Chlorella vulgaris* (*C.vulgaris*) were provided by bulk supplements in Henderson, USA. The *C. vulgaris* biomass composition was analyzed using chromatography in the research center and food pollution/environment and water research at the Ministry of Science and Technology. The methodology which was given by (Ramluckan, Moodley, and Bux 2014) for the recovery of lipid and chlorophyll from non-living microalgae was adopted in this study with some modifications. The current study employed the soxhlet apparatus to extract oil and chlorophyll from microalgae biomass. This apparatus is frequently used for compound extraction when the solvent's solubility level is low and the impurities become soluble in the solvent. The required solute was extracted from the treated material in the solvent extraction procedure after a prolonged interaction between the sample and the solvent. About 20 g of dried *C. vulgaris* powder was correctly weighed and added to the device's thimble; the thimble was composed of filter paper with a diameter of 5 cm and a thickness of 3 mm. The extraction chamber of the soxhlet apparatus received the thimble and moved it there. A 600 mL
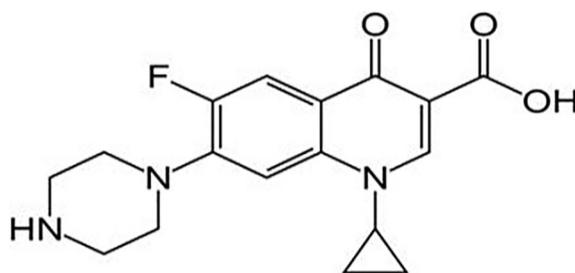
Figure 1. The structure of Ciprofloxacin

aliquot of the extraction solvent was placed on the heating plates in the flask. The condenser's cooling water supply was opened to ensure the solvent's continual recycling. The solvent was heated to a boiling point using an electric heater. The boiling points of the solvents listed in the table1 were used to program the boiling point temperatures. When the solvent vapor starts to condense in the reaction chamber, the reaction produces the microalgae content – solvent mixture occurs after the algae powder and the solvent has been fully mixed. A siphon system arrangement was used to empty the solvent-containing solutes into the flask. After the extraction, the samples were allowed to cool for at least 15 minutes before removing the solvent flask containing the components extracts. In order to extract the majority of components present in the biomass and produce an adsorbent with a high ability to adsorb the contaminants, this procedure was repeated twice for the same amount of biomass using two different solvents (ethanol and chloroform), with time-based extractions performed hourly between 1 and 10 hours for each process.

Preparation of biosorbent

After the extraction procedure, the leftover *C. vulgaris* biomass was collected. Then rinse it with deionized water. To remove unreacted solvents (chloroform and methanol), centrifuging tubes containing *C. vulgaris* biomasses were filled with deionized and shaken the tubes for five minutes. The microalgal biomasses were spun using a centrifuge apparatus (CAPP, CR-656), and the supernatant was discarded. This method was repeated several times. Finally, microalgal biomasses were centrifuged and dried in a 40 °C oven before being crushed and utilized. Ciprofloxacin was removed from water using adsorbents produced from powdered algal masses (with no further chemical or physical modification).

Adsorption experiment

Evaluation of CIP removal from water by microalgal biomasses was conducted by batch biosorption tests. Many factors, including sorbent concentration, starting adsorbate concentration, contact time, pH, agitation rate, and temperature, play a role in the biosorption procedure (Crini et al., 2008). Initial CIP concentration (50 to 450) mg/L, adsorbent quantity (0.5 to 5.0) g/L,the

effects of initial CIP solution pH (3.0 to 11.0), and contact time (10–240 min) were studied by biosorption studies. To determine the equilibrium period, adsorption trials were conducted on 50 mL of CIP solution (450 mg/L) with a 2 g/L dosage at constant temperature for 240 minutes,with containers shaken at 200 rpm. 10 mL sample was centrifuged and then filtered using 0.2 μm membrane filters to isolate the adsorbent from the aqueous phase. The residual CIP concentration was detected by chromatography HPLC model SYKAMN in the research center and food pollution/environment and water research at the Ministry of Science and Technology. It was equipped with an injection valve of 100 μl, detector U.V./Vis set at 278 nm, and reverse phase analytical column C18 (25 cm × 4.6 mm × 3.5 μm) The mobile phase used was gradually a mixture (A = 0.025 M phosphoric acid adjusted to pH 3 with triethanolamine) and B = acetonitrile, C = methanol (40:40:20) at a flow rate of 1.0 ml/min.

According to Eq. (1), one gram of adsorbent has a capacity (*q*) for Ciprofloxacin equal to the amount of Ciprofloxacin bound to it in milligrams (Hammud et al., 2011).

$$q = \left(\frac{mg}{g}\right) = \frac{(C_i - C_e)}{m} \times v \qquad (1)$$

The percentage removal of Ciprofloxacin was calculated according to Eq. (2)

$$\%R = \frac{C_i - C_e}{C_i} \times 100 \qquad (2)$$

where: *m* – the adsorbent mass (g);

V – the volume of ciprofloxacin solution (L) in contact with the sorbent;

C_i and C_e – the initial and equilibrium ciprofloxacin concentrations (mg/L), respectively.

Characterization of *C. vulgaris*

FTIR

Spectra of *C. vulgaris* biomass were captured using an (FTIR) spectrometer (FTIR (ATR), 1800, SHIMADZU, Japan). The spectra were taken with a KBr disc between 400 and 4000 cm[1] in wavelength. This analytical technique was applied to a *C. vulgaris* biomass sample to identify the functional group(s) on its outermost surface.

Table 1. Solvents used for soxhlet extractions showing their relevant properties in order of increasing polarity index

Solvent	Polarity index Units	Boiling point C°	Density@25C° g/ml
Ethanol	5.2	78.0	0.789
Chloroform	4.1	60.5_61.5	1.492

Note: data courtesy of Aldrich handbook of fine chemicals, 2009–2010 (Ramluckan et al., 2014).

Surface area

The surface area of the adsorbents was measured using the BET method (BELSORP MINI II, BEL, Japan) based on nitrogen adsorption-desorption isotherms at 77 K.

SEM analyses and EDX

The morphology of the microalgal bio adsorbent was examined using the scanning electron (SEM) microscope (FESEM-EDS, MIRA III, TESCAN, Czech). Additionally, the elemental analyzer (Xflash 6110, Burkercompany, Germany) was used to determine the elemental composition of the bio adsorbent.

X-ray

The crystal structure of the adsorbentbiomass was determined using the XRD pattern (PW1730, Philips, Holanda) with Cu Kα radiation (λ = 0.1542 nm) in 2θ angle range of 0–70°.

RESULTS AND DISCUSSION

Biochemical composition of microalgal biomass

The microalgae were biochemically characterized in all of their components. The goal wasto provide foundational knowledge for targeted and optimized downstream processing. The biochemical composition of microalgal biomasses is described in Table 2. Biomass from *C. vulgaris* is primarily composed of proteins. Protein concentration is as high as 55%, with fat at 6.25% and ash at 7.58, comparably close to (Tokuşoglu, 2003). also, *C. vulgaris* has a lot of minerals like calcium, zinc, iron, copper, and magnesium (Mathys et al., 2021).

Enhancement of CIP's biosorption

A preliminary experiment was carried out for CIP adsorption on unextracted *C. vulgaris* biomass; it was observed that only 20% of CIP was

Table 2. Biochemical composition of microalgal biomass

No	Name	Con
1	Protein %	55.8
2	Lipid %	6.25
3	Ash %	7.58
4	Moisture %	9.55
5	CHO %	20.82
6	Chlorophyll (mg/100 gm)	9.82
7	Ca (mg/gm)	250
8	Fe (mg/gm)	180
9	Cu (mg/gm)	50
10	Zn (mg/gm)	177
11	Mg (mg/gm)	182

removed. However, extracted *C. vulgaris* biomass removed CIP at a rate of 89.9%. As a result, the following elements influencing the adsorption process were investigated.

Characterization of the algal biosorbent

It is generally known that the structural properties of an adsorbent material determine its performance in adsorption operations (Nguyen et al., 2013). The essential structural properties of an adsorbent are determined by the amount and kind of superficial functional groups and the porosity of the surface. Suppose the number of functional groups on the surface is high and the surface is porous. In that case, the material under study can be utilized in the removal process of CIP.

FTIR studies

The FTIR spectra of *C. vulgaris* biomass before extraction revealed multiple strong and intense bands with vibrational frequencies of 3313, 2924, 1654, and 1543 cm^{-1} (Fig. 2a). The high broadband at 3313 cm^{-1} indicates the presence of the -O.H. and -N.H. groups. This peak shows the possibility of alcoholic, amide and phenolic chemicals occurring in the species (Nithya et al., 2019). The –C.H. stretch corresponds to the band at 2924 cm^{-1} within the range 2809–3012 and was characterized by strong vibrations in lipids and

carbohydrates. The protein spectra of Chlorella Vulgaris are distinguished by strong peaks in the 1654 cm^{-1} (amide I) range (1583–1709) and 1543 cm^{-1} (amide II) range (1481–1585). In amide complexes, these bands were caused primarily by C=O stretching vibrations and a combination of N-H and C-H stretching vibrations (Indhumathi et al., 2013). C-C stretching of aromatic, i.e., in the ring, is attributed to the 1400–1500 cm^{-1} range, while C–N stretching of aliphatic amine tumbled in the
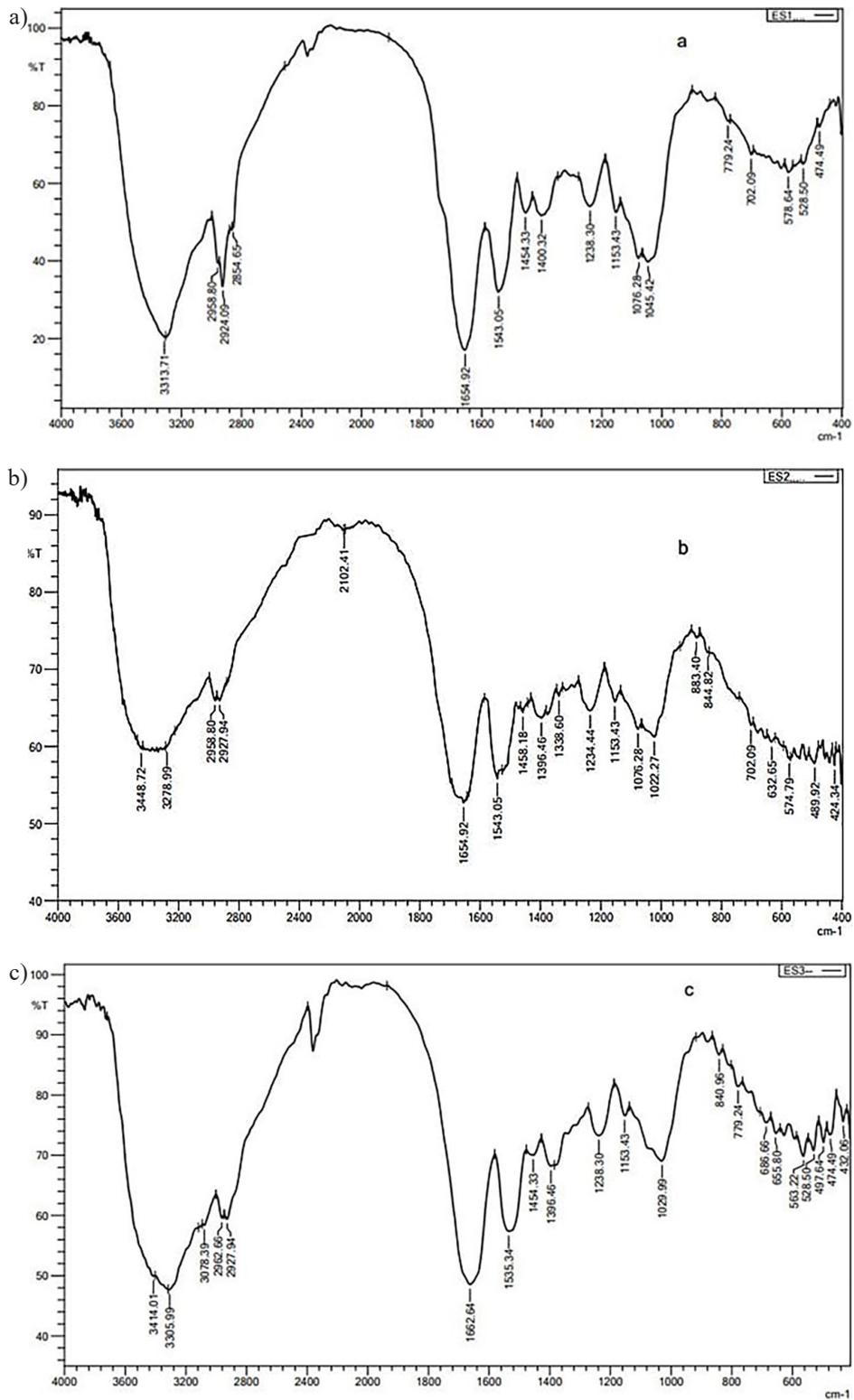
Figure 2. FTIR analysis of raw *C. vulgaris* biomass (a), extracted *C. vulgaris* biomass (b), andextracted biomass to that of the CIP-loaded (c)

1250–1020 cm^{-1} range. Carbohydrates are indicated by a C-O-C, C-O stretching between 1000 and 1200 cm^{-1} (Mishra et al., 2009). The peaks at 1080 cm^{-1} and 1240 cm^{-1} frequencies could be attributed to the symmetric and asymmetric PO$_2^-$, which demonstrated stretch (Nithya et al., 2019). The peak at 528 has a relationship to C-Cl (500–550 cm^{-1}) (Elgarahy et al., 2020). From what has been said above, we can infer that *Chlorella* is dominated by hydroxyl, amide, carboxylate, phosphoryl, nitrate, and phenolic groups. Figure 2b also shows the FTIR analysis of *C. vulgaris* after it has been extracted. Changing band wavenumbers are used to identify the extracted *C. vulgaris*' functional groups. The significant groups' impact was also calculated by watching for the emergence of new bands and the disappearance of previous peaks. Most noticeably, the peak of the -O.H. and -N.H. groups has moved, with the vibrational frequency now spanning a broad range from 3278 to 3448 cm^{-1}, down from 3313. The amide I and amide II peaks, at 1654 and 1543 cm^{-1}, respectively, have not moved, suggesting that the functional groups have grown as a result of the extraction rather than shrunk (Nithya et al., 2019). Figure 2c depicts the Ciprofloxacin absorption by the host's biomass from the solution. Comparing the FTIR data of the extracted biomass to that of the CIP-loaded extracted biomass residue reveals that the peaks of certain functional groups have shifted toward lower energies. Attaching CIP to a functional group of organic compounds causes a drop in electron density and an increase in bond length by attracting the functional group's electron cloud (Elangovan, Philip, and Chandraraj, 2008).

BET

After the extraction process, the biomass's BET surface area increased dramatically, going from 2.3723 to 3.6224 m^2/g. Disruption of cells during extraction may account for these elevations (Xie et al., 2014). generally classifies pores as either micro-pores (those smaller than 2.0 nm), mesopores (those between 2.0 and 50 nm), or macro-pores (those larger than 50 nm)(Li-Jian Leng et al. 2015). Extracted. C.Vulgaris generated in this work were classified as mesopore materials since their typical pore sizes ranged from 2.0 to 50 nm, as shown in Table 3. The BET was analyzed for the extracted biomass that had been loaded with CIP. The decreased surface area of the CIP-loaded, extracted biomass reflected the level of occupancy (Pradhan et al., 2019).

SEM analyses and EDX

Figure 3 (A, CandE) is a scanning electron micrograph (SEM) comparison of unprocessed, extracted, and loaded CIP-extracted biomass. A look at Figure 3A Unprocessed biomass has cells that are uniform in size, shape, and distribution (Mahmood et al., 2017). Figure 3C shows that the extracted biomass appears to have been broken down, as the cells are contracted and wrinkled. This suggests that the intercellular matter has been exposed to the environment and has come into contact with the extraction solvents (Mahmood et al., 2019). Fig. 3E depicts the biomass extracted following the adsorption process. Loaded particles are visible throughout the image due to the CIP effect, with a close, compact, and smoother structure (Kumar, Singh, and Sikandar, 2020). The primary components in biomass are identified in the EDX spectrum of the raw. C.Vulgaris extracted C.Vulgaris and loaded CIP-extracted biomass surfaces. These nutrient components include carbon, Nitrogen, phosphorus, calcium, magnesium, and oxygen. All three biomass states contained these components (Kumari et al., 2019). Elements analyzed in extracted algal cells show a slight decrease in C and other components. Given these findings, it appears that the majority of the lipids were successfully removed

Table 3. Characteristics of raw *C. vulgaris*, after the extraction process and extracted *C. vulgaris*

Characteristics	Biomass before extraction	Biomass after extraction	Loaded CIP - extracted biomass
Surface area (m^2/g)	2.3723	3.6224	2.7969
Average pore diameter (nm)	15.788	10.058	11.64
C(%)	63.5	61.73	55.52
N(%)	26	28.1	27.5
O(%)	21.1	18.22	22.7
P(%)	2.43	1.54	2.6
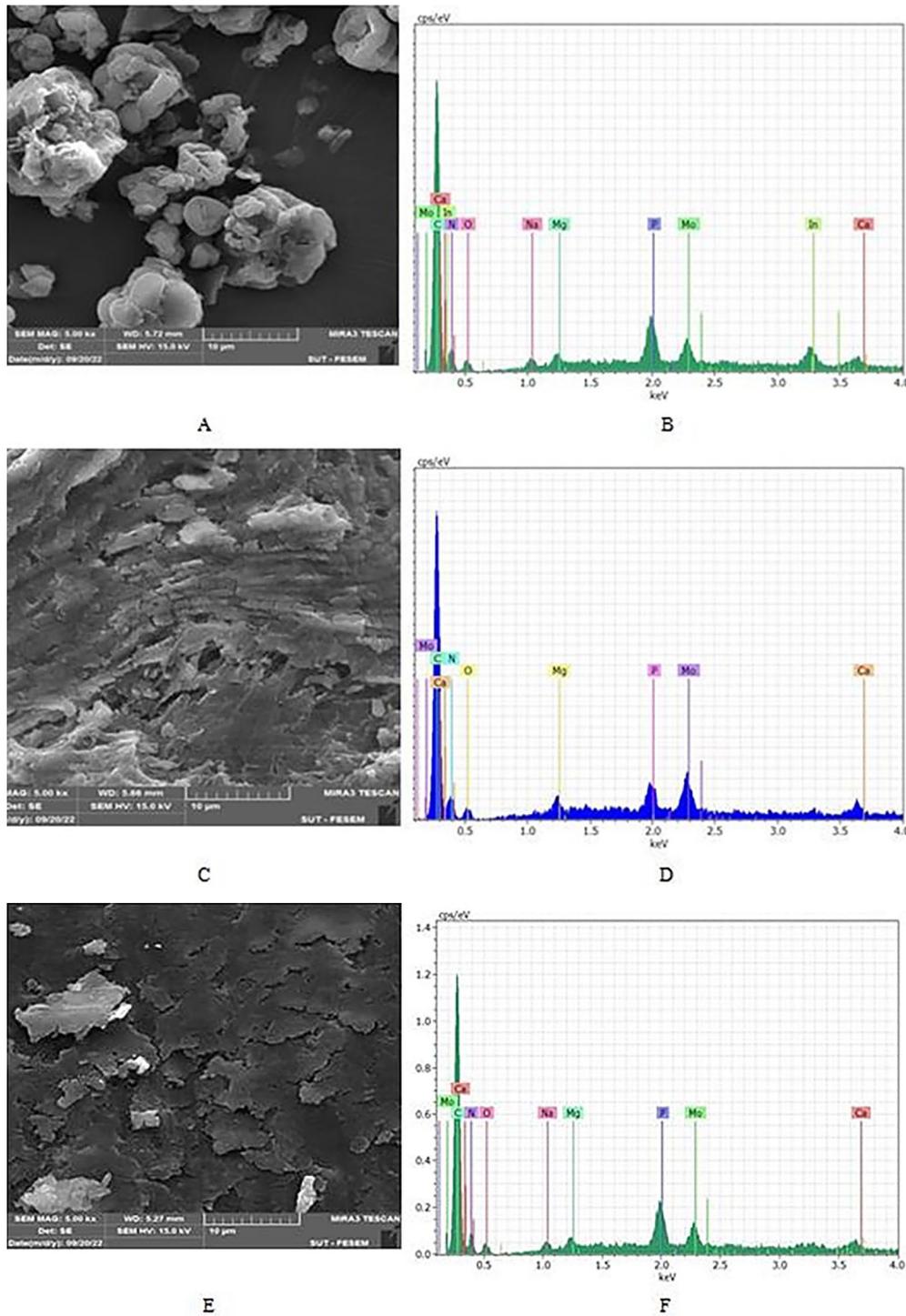
Figure 3. SEM-EDX spectra of *C. vulgaris* biomass, (a) and (b) before extraction;
(c) and (d) afterthe extraction; (e) and (f) after being loadedwith CIP

and eliminated in (Table 4) (Anastopoulos et al., 2015). Elements were most likely ion-exchanged with CIP following biosorption, resulting in a shift in elemental ratios (Oliveira et al., 2014).

XRD patterns

Figure 4a shows that the raw biomass of *C. vulgaris* microalgae measured 19.65 and 28.065

at 2Θ degrees, with d-spacings of 4.51 and 3.17, respectively. Figure 4b shows that the extracted microalgal biomass of *C. Vulgaris* measured 17.78 and 27.7 at 2Θ degrees, with d-spacings of 4.98 and 3.21, respectively, due to the amorphous carbon structure in the microalgae structure (Bekirogullari, Kaya, and Saka 2019; Dalal et al. 2021). The intensity and width of the peaks at 2Θ = 17.78 and 27.7 were
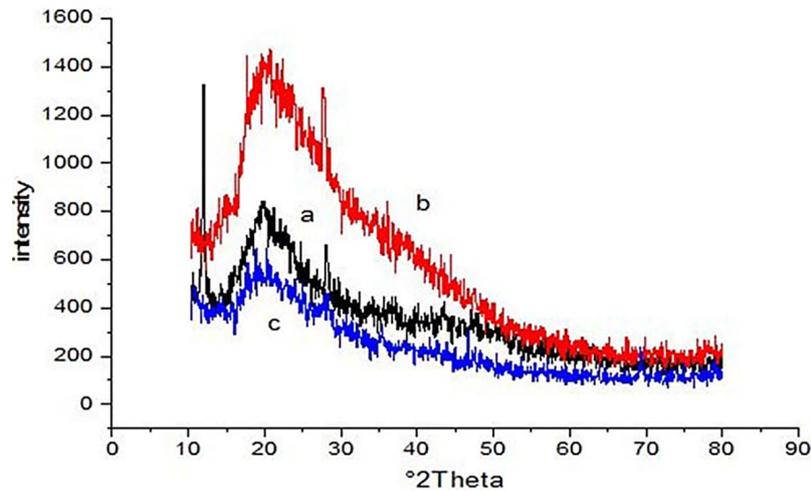
Figure 4. The raw *C. vulgaris* microalgal strain (a), extracted *C. vulgaris* microalgal biomass (b), and Ciprofloxacin-loaded extracted-*C. vulgaris* microalgal biomass (c)

both enhanced in Figure 4b, which demonstrated the destruction of the more amorphous region (Wu et al. 2019). Figure 4c is displayed. Ciprofloxacin adsorption on the extracted C.Vulgaris biomass surface resulted in a deviation of the XRD profile to 2Θ = 69.29 with d-spacings of 1.35499, indicating that the functional group and crystalline nature were altered (Yuan et al., 2010; Narayan et al., 2016).

Effect of contact time

Batch biosorption experiments were used to conduct time course studies on the CIP biosorption for solutions containing (50, 150, and 450 mg/L) of biosorbent with a dose of 2 g/L. According to Figure 5, which shows a link between CIP adsorption capacity and adsorption period, CIP adsorption capacity clearly increased throughout the first 120 minutes of the CIP-biosorbent contact. This behavior may be explained by the instantaneous use of the adsorbing sites that are most easily accessible on the adsorbent surface (Yang et al., 2010). Thereafter, the adsorption rate decreased because the remaining surface site was difficult to occupy due to the electrostatic repulsive forces between the CIP molecules and the surface (Ojedokun et al., 2017; Agboola et al., 2020). Adsorption equilibrium was reached when the rate of Ciprofloxacin adsorption onto the adsorbent was equal to the rate of desorption from the adsorbent. At this point, the adsorption process stabilized, and the amount adsorbed was roughly constant (Zhang et al., 2011). The contact time curve analysis showed that 120 minutes was the best time for all concentrations of Ciprofloxacin. This explains why 120 minutes of interaction time was found to be optimal in batch equilibrium trials.
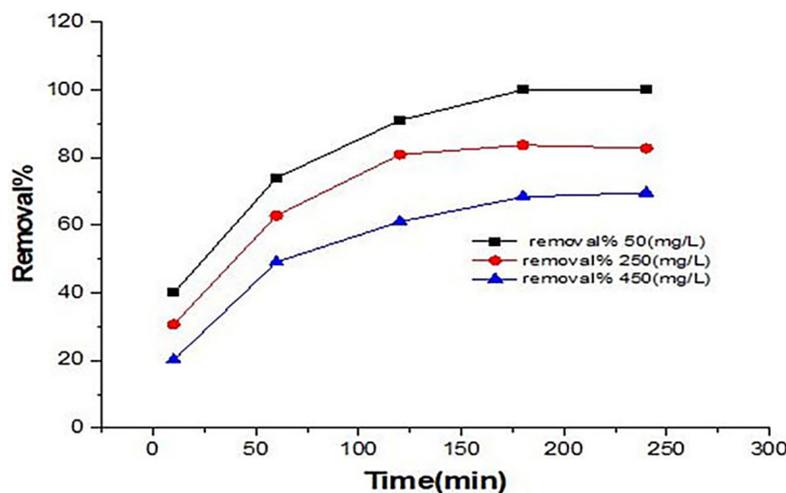
Figure 5. Effect of contact time on the biosorption of ciprofloxacin, biosorbentdose: 2 g/L;PH:7, ciprofloxacin concentration (50, 250,450 mg/L)

Adsorbent dosage response

Knowing the adsorbent dosage at which maximal adsorption occurs is crucial. As a result, adsorption research is carried out utilizing a range of adsorbent loadings. The tests were carried out for 450 mg/LCIP solution at pH 7. The contact time and temperature of the CIP solution are kept at 120 minutes and room temperature, respectively. Figure 6 shows the efficiency of extracting *C. vulgaris* biomass to remove CIP at different concentrations ranging from 0.5 to 5.0 g/L.The efficiency of CIP removal rose dramatically from 69.16% to a maximum of 89.92% when the adsorbent quantity increased from 0.5 to 2.75 g/L.Increasing the adsorbent dose results in a higher removable percentage because a larger adsorbent's surface area and pore capacity can house more functional groups and active adsorption sites (Ahmed et al., 2014; Peñafiel et al., 2019). However, removal efficiency declined gradually and steadily as the absorbent concentration increased from 2.75 to 5 g/L. The removal of CIP was reduced, which could be attributable to adsorptionsite overlapping or aggregation, leading to a decrease in the total adsorbent surface (Javed et al., 2007; S et al., 2016).

The effect of solution pH-

The optimal pH is critical because it affects not only the surface charge of the adsorbent but also the degree of ionization and speciation of the adsorbate throughout the reaction (Babel et al., 2004; Ruiying et al., 2007). CIP has two pKa values: 5.90±0.15 for the carboxylic acid group and 8.89±0.11 for the basic-Nmoiety (Yan et al., 2017). When CIP is in its cation form, its acid dissociation constant, pKa1, is less than 6.0 due to the protonation of the amine group, and when it is in its anion form, its acid dissociation constant, pKa2, is greater than 8.7 due to loss of a proton from the carboxylic group. Zwitterionic species of CIP molecules predominate in the range of 6.0–8.7 pH. Deprotonation of the carboxylic acid in the aqueous solution group to the negatively charged carboxylate since it is above the pKa1 of the carboxylic group and still below the pKa2 of the amine group, keeping the amine group protonated and positively charged (Jiang et al., 2013). Experiments were carried out at varied beginning pH values (3.0–11.0) and at room temperature with a two hours optimization time to determine the best-suited pH for the successful biosorption of CIP on waste *C. vulgaris* biomass. Figure 7 illustrates that an increase in pH from 3 to 7 resulted in better adsorption of CIP. It is possible that hydrophobic interactions between functional groups on the waste surface of *C. vulgaris* and CIP are responsible for the biosorption mechanism. Acidic/neutral mediums are preferred by the adsorption process when the pKa of CIP is between (6–8.7). At higher pH values, electrostatic repulsion between adsorbents and CIP molecules is expected to limit biosorption because CIP molecules are negatively charged, and biomass is also negatively charged closely (Ali et al., 2018).
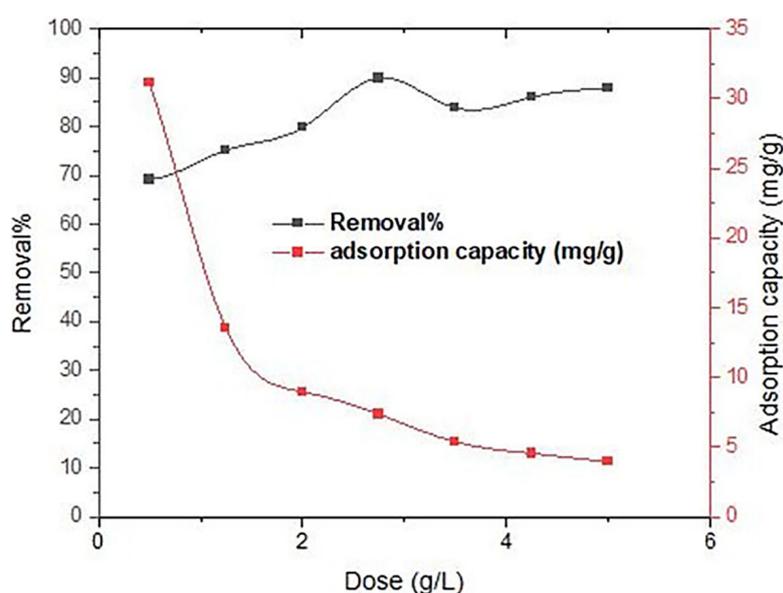
Figure 6. Effect of biosorbent dosage on the biosorption of ciprofloxacin, contact time 120 min; pH=7, ciprofloxacin concentration 450 mg/L
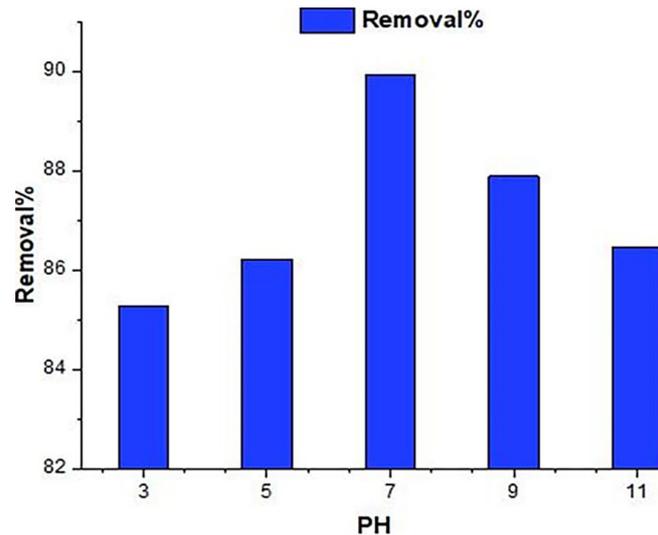
Figure 7. Effect of PH on the biosorption of ciprofloxacin,contact time 120 min.; biosorbent dose: 2 g/L;ciprofloxacin concentration 450 mg/L

Effect of initial concentration

As shown in Figure 8, the removal effectiveness of the adsorbate (Ciprofloxacin) utilizing the adsorbent (extracted *C. vulgaris*) is affected by the starting adsorbate concentration. Biosorption of CIP by *C. vulgaris* biomass was studied with an initial CIP ion concentration spanning 50 to 450 mg/L, the adsorbent dosage of 2 g/L. P.H.; 7 and contact time 120 min. A look at Figure 8 reveals that as the starting concentration is increased, the adsorption effectiveness decreases. This pattern can be explained by the fact that adsorbents have a fixed, finite number of active sites accessible for adsorption and hence become saturated at significant concentrations. Furthermore,

it was founded that greater initial CIP concentrations resulted in larger adsorption capacities as a result of the increase in the driving force, or concentration gradient of adsorption, to overcome the CIP transfer's resistance between the aqueous and solid phases (Yang et al., 2010). It's possible that the decrease in adsorption efficiency is due to insufficient surface area to deal with significantly higher CIP in the solution. Moreover, It was postulated that a higher percentage of adsorption would occur at lower CIP ion concentrations because all of the CIP ions in solution may engage with the binding sites, while the adsorption yield decreases with increasing concentration because the available adsorption sites are eventually filled as proven (Kumar et al., 2006).
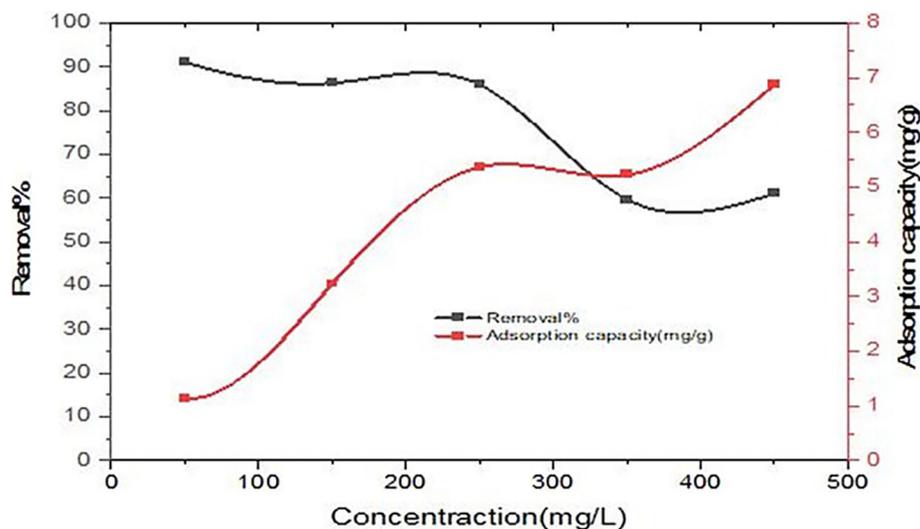
Figure 8. Effect of initial concentration of Ciprofloxacin, pH=7, adsorbent dose: 2 g/L; contact time 120 m

CONCLUSIONS

The adsorption of Ciprofloxacin onto waste *C. vulgaris* biomass was researched to determine the usefulness of low-cost biomass for the removal of contaminants from an aqueous medium. The biosorption of Ciprofloxacin is sensitive to changes in contact time, pH, and adsorbent dose. High removal efficiency (89.9%) was achieved when Ciprofloxacin was removed from aqueous solutions using *C. vulgaris* biomass, 2.75 g/L adsorbents, 120 minutes of contact time, and a pH of 7. After lipid extraction, the biomass BET surface area increased dramatically, going from 2.3723 to 3.6224 m^2/g. The present investigation reveals that the adsorption process retains several of the important functional groups of waste *C. vulgaris*, including amide and carboxylic groups. Amorphous carbon was confirmed by X-ray diffraction patterns, and elemental distributions such as carbon, Nitrogen, oxygen, and phosphorus were measured by EDX. Waste microalgae adsorbents can be employed as environmentally acceptable adsorbents for the removal of antibiotics from water due to their strong adsorption capabilities.

REFERENCES

1. Abbas A.S.,Darweesh T.M. 2016. Preparation and Characterization of Activated Carbon for Adsorption of Fluoroquinolones Antibiotics. Journal of Engineering, 22, 140–157.

2. Acero J.L.,Benitez F.J.,Leal A.I.,Real F.J.,Teva F. 2010. Membrane filtration technologies applied to municipal secondary effluents for potential reuse. Journal of Hazardous Materials.

3. Agboola O.S.,Bello O.S. 2020. Enhanced adsorption of ciprofloxacin from aqueous solutions using functionalized banana stalk. Biomass Conversion and Biorefinery, 12, 5463–5478.

4. Ahmed M.J.,Theydan S.K. 2014. Fluoroquinolones antibiotics adsorption onto microporous activated carbon from lignocellulosic biomass by microwave pyrolysis. Journal of the Taiwan Institute of Chemical Engineers, 45, 219–226.

5. Ahmed M.J.,Hameed B.H.,Hummadi E.H.2020. Review on recent progress in chitosan/chitin-carbonaceous material composites for the adsorption of water pollutants. Carbohydrate Polymers, 247, 116690.

6. Ali M.E.M.,El-aty A.M.A.,Badawy M.I.,Ali R.K.2018. Removal of pharmaceutical pollutants from synthetic wastewater using chemically modified biomass of green alga Scenedesmus obliquus. Ecotoxicology and Environmental Safety, 151, 144–152.

7. Anastopoulos I.,Kyzas G.Z. 2015. Progress in batch biosorption of heavy metals onto algae. Journal of Molecular Liquids, 209, 77–86.

8. Aristilde L.,Melis A.,Sposito G. 2010. Inhibition of photosynthesis by a fluoroquinolone antibiotic. Environmental Science and Technology.

9. Audenaert W.T.M.,Vandierendonck D.,Van Hulle S.W.H.,Nopens I. 2013. Comparison of ozone and HO induced conversion of effluent organic matter (EfOM) using ozonation and UV/H2O2 treatment. Water Research.

10. Babel S.,Kurniawan T.A. 2004. Cr(VI) removal from synthetic wastewater using coconut shell charcoal and commercial activated carbon modified with oxidizing agents and/or chitosan. Chemosphere.

11. Buerge I.J.,Poiger T.,Müller M.D.,Buser H.R. 2003. Caffeine, an anthropogenic marker for wastewater contamination of surface waters. Environmental Science and Technology.

12. Crini G.,Badot P.M. 2008. Application of chitosan, a natural aminopolysaccharide, for dye removal from aqueous solutions by adsorption processes using batch studies: A review of recent literature. Progress in Polymer Science (Oxford).

13. Dalal S.R.,Hussein M.H.,El-Naggar N.E.,Mostafa S.I.,Shaaban-Dessuuki S.A. 2021. Characterization of alginate extracted from Sargassum latifolium and its use in Chlorella vulgaris growth promotion and riboflavin drug delivery. Scientific Reports, 11, 1–17.

14. De la Cruz N.,Giménez J.,Esplugas S.,Grandjean D.,De Alencastro L.F.,Pulgarín C. 2012. Degradation of 32 emergent contaminants by UV and neutral photo-fenton in domestic wastewater effluent previously treated by activated sludge. Water Research.

15. Deziel N. 2014. Pharmaceuticals in Wastewater Treatment Plant Effluent Waters. University of Minnesota, Morris.

16. Dijkshoorn L.,Nemec A.,Seifert H. 2007. An increasing threat in hospitals: Multidrug-resistant

Acinetobacter baumannii. Nature Reviews Microbiology.

17. Elangovan R.,Philip L.,Chandraraj K. 2008. Biosorption of hexavalent and trivalent chromium by palm flower (Borassus aethiopum). Chemical Engineering Journal.

18. Elgarahy A.M.,Elwakeel K.Z.,Mohammad S.H.,Elshoubaky G.A. 2020. Multifunctional eco-friendly sorbent based on marine brown algae and bivalve shells for subsequent uptake of Congo red dye and copper(II) ions. Journal ofEnvironmental Chemical Engineering, 8.

19. Gimeno O.,García-Araya J.F.,Beltrán F.J.,Rivas F.J.,Espejo A. 2016. Removal of emerging contaminants from a primary effluent of municipal wastewater by means of sequential biological degradation-solar photocatalytic oxidation processes. Chemical Engineering Journal.

20. Githinji L.J.M.,Musey M.K.,Ankumah R.O. 2011. Evaluation of the fate of ciprofloxacin and amoxicillin in domestic wastewater. Water, Air, and Soil Pollution.

21. Gupta V.K.,Carrott P.J.M.,Ribeiro Carrott M.M.L.,Suhas. 2009. Low-Cost adsorbents: Growing approach to wastewater treatmenta review. Critical Reviews in Environmental Science and Technology, 39, 783–842.

22. Gupta V.K.,Saleh T.A. 2013. Sorption of pollutants by porous carbon, carbon nanotubes and fullerene-An overview. Environmental Science and Pollution Research, 20, 2828–2843.

23. Hammud H.H.,Fayoumi L.,Holail H.,Mostafa E.S.M.E. 2011. Biosorption Studies of Methylene Blue by Mediterranean Algae Carolina and Its Chemically Modified Forms. Linear and Nonlinear Models' Prediction Based on Statistical Error Calculation. International Journal of Chemistry.

24. Han R.W.,Zheng N.,Yu Z.N.,Wang J.,Xu X.M.,Qu X.Y.,Li S.L.,Zhang Y.D.,Wang J.Q. 2015. Simultaneous determination of 38 veterinary antibiotic residues in raw milk by UPLC-MS/MS. Food Chemistry.

25. Hirsch R.,Ternes T.,Haberer K.,Kratz K.L. 1999. Occurrence of antibiotics in the aquatic environment. Science of the Total Environment.

26. Indhumathi P.,Soundararajan M.,Shabudeen P.S.S.,Suresh E.S.A. 2013. Utilization, isolation and characterization of chlorella vulgaris for carbon sequestration and waste water treatment by performing ftir spectral studies. Asian Jr. of Microbiol. Biotech. Env. Sc., 15, 661–666.

27. Irem S.,Mahmood Khan Q.,Islam E.,Jamal Hashmat A.,Anwar ul Haq M.,Afzal M.,Mustafa T. 2013. Enhanced removal of reactive navy blue dye using powdered orange waste. Ecological Engineering, 58, 399–405.

28. Javed M.A.,Bhatti H.N.,Hanif M.A.,Nadeem R. 2007. Kinetic and Equilibrium Modeling of Pb(II) and Co(II) Sorption onto Rose Waste Biomass. Separation Science and Technology, 42, 3641–3656.

29. Jiang W.T.,Chang P.H.,Wang Y.S.,Tsai Y.,Jean J.S.,Li Z.,Krukowski K. 2013. Removal of ciprofloxacin from water by birnessite. Journal of Hazardous Materials.

30. Karthikeyan K.G. 2005. Sorption of the Antimicrobial Ciprofloxacin To Aluminum and Iron Hydrous Oxides. Environmental Science, Engineering, and Technology, 39, 9166–9173.

31. Kelly B.C.,Ikonomou M.G.,Blair J.D.,Morin A.E.,Gobas F.A.P.C. 2007. Food web-specific biomagnification of persistent organic pollutants. Science.

32. Klavarioti M.,Mantzavinos D.,Kassinos D. 2009. Removal of residual pharmaceuticals from aqueous systems by advanced oxidation processes. Environment International, 35, 402–417.

33. Kumar M.,Singh A.K.,Sikandar M. 2020. Biosorption of Hg (II) from aqueous solution using algal biomass: kinetics and isotherm studies. Heliyon, 6.

34. Kumar Y.P.,King P.V.S.R.K.P. 2006. Zinc biosorption on Tectona grandis L.f. leaves biomass: Equilibrium and kinetic studies. Chemical Engineering Journal 124., 124, 63–70.

35. Kumari N.,Singh R.K. 2019. Biofuel and co-products from algae solvent extraction. Journal of Environmental Management., 247, 196–204.

36. Kümmerer K. 2009. Chemosphere Antibiotics in the aquatic environment – A review – Part I. Chemosphere, 75, 417–434.

37. Lapworth D.J.,Baran N.,Stuart M.E.,Ward R.S. 2012. Emerging organic contaminants in groundwater : A review of sources, fate and occurrence. Environmental Pollution, 163, 287–303.

38. Leng L.,Yuan X.,Zeng G.,Shao J.,Chen X.,Wu Z.,Wang H.,Peng X. 2015a. Surface characterization of rice husk bio-char produced by liquefaction and application for cationic dye (Malachite green) adsorption. Fuel.

39. Leng L. Jian,Yuan X.Z.,Huang H.J.,Wang H.,Wu Z.B.,HuanF.L.,Peng X., HongC.X.,MingZ.G. 2015b. Characterization and application of biochars from liquefaction of microalgae, lignocellulosic biomass and sewage sludge. Fuel Processing Technology, 129, 8–14.

40. Li Y.,Horsman M.N.W.,Lan C.Q.Nathalie Dubois-Calero, 2008. Biofuels from Microalgae. Biotechnology Progress, 24, 277–321.

41. Lindsey M.E.,Meyer M.,Thurman E.M. 2001. Analysis of trace levels of sulfonamide and tetracycline antimicrobials in groundwater and surface water using solid-phase extraction and liquid

chromatography/mass spectrometry. Analytical Chemistry.

42. Mahmood W.M.A. W.,Theodoropoulos C.,Gonzalez-Miquel M., 2017. Enhanced microalgal lipid extraction using bio-based solvents for sustainable biofuel production. Green Chemistry, 19, 1–13.

43. Mahmood W.M.A.W., Lorwirachsutee A., Theodoropoulos C., Gonzalez-Miquel M. 2019. Polyol-based deep eutectic solvents for extraction of natural polyphenolic antioxidants from Chlorella vulgaris WAN. ACS Sustainable Chem. Eng., 7, 5018–5026.

44. Marchal G.,Smith K.E.C.,Rein A.,Winding A.,Trapp S.,Karlson U.G. 2013. Comparing the desorption and biodegradation of low concentrations of phenanthrene sorbed to activated carbon, biochar and compost. Chemosphere, 90, 1767–1778.

45. Mata T.M.,Martins A.A.,Caetano N.S. 2010. Microalgae for biodiesel production and other applications: A review. Renewable and Sustainable Energy Reviews., 14, 217–232.

46. Mathys A.,Canelli G.,Martínez P.M.,Austin S.,Ambühl M.E.,Dionisi F.,Bolten C. J.,Carpine R.,Neutsch L. 2021. Biochemical and Morphological Characterization of Heterotrophic Cryptheco-dinium cohnii and Chlorella vulgaris Cell Walls. Journal of Agricultural and Food Chemistry.

47. Mishra A.,Jha B.2009. Isolation and characterization of extracellular polymeric substances from micro-algae Dunaliella salina under salt stress Avinash. Bioresource Technology.

48. Mohammed-Ridha M.J.,Abdul-Ahad M.Y. 2014. Adsorption of Levofloxacine Antibacterial from Contaminated Water by Non – Conventional Low Cost Natural Waste Materials. Journal of Engineering, 20, 88–104.

49. Möller P.,Dulski P.,Bau M.,Knappe A.,Pekdeger A.,Sommer-Von Jarmersted C. 2000. Anthropogenic gadolinium as a conservative tracer in hydrology. Journal of Geochemical Exploration.

50. Narayan R.,Meena R.P.,Patel A.K.,Prajapati A.K.,Srivastava S.,Mondal M.K. 2016. Characterization and Application of Biomass Gasifier Waste Material for Adsorptive Removal of Cr (VI) from Aqueous Solution Ram. Environmental Progress & Sustainable Energy, 35, 95–102.

51. Nguyen T.A.H.,Ngo H.H.,Guo W.S.,Zhang J.,Liang S.,Yue Q.Y.,Li Q.,Nguyen T.V. 2013. Applicability of agricultural waste and byproducts for adsorptive removal of heavy metals from wastewater. Bioresource Technology.

52. Nithya K.,Sathish A.,Pradeep K.,Kiran Baalaji S. 2019. Algal biomass waste residues of Spirulina platensis for chromium adsorption and modeling studies. Journal of Environmental Chemical Engineering.

53. Ojedokun A.T.,Bello O.S. 2017. Kinetic modeling of liquid-phase adsorption of Congo red dye using guava leaf-based activated carbon. Applied Water Science.

54. Oliveira R.C.,Hammer P.,Guibal E.,Taulemesse J.M.,Jr. O.G. 2014. Characterization of metal–biomass interactions in the lanthanum(III) biosorption on Sargassum sp. using SEM/EDX, FTIR, and XPS: Preliminary studies. Chemical Engineering Journal, 239, 381–391.

55. Peñafiel M.E.,Vanegas E.,Bermejo D.,Matesanz J.M.,Ormad M.P. 2019. Organic residues as adsorbent for the removal of ciprofloxacin from aqueous solution. Hyperfine Interact., 240.

56. Picó Y.,Andreu V. 2007. Fluoroquinolones in soil-risks and challenges. Analytical and Bioanalytical Chemistry.

57. Pradhan A.,Sukla L.B.,Mishra B.B.,Devi N. 2019. Biosorption for removal of hexavalent chromium using microalgae Scenedesmus sp. Journal of Cleaner Production, 209, 617–629.

58. Ramanan R.,Kim B.H.,Cho D.H.,Oh H.M.,Kim H.S. 2016. Algae-bacteria interactions: Evolution, ecology and emerging applications. Biotechnology Advances.

59. Rodriguez-Mozaz S.,Ricart M.,Köck-Schulmeyer M.,Guasch H.,Bonnineau C.,Proia L.,de Alda M.L.,Sabater S.,Barceló D. 2015. Pharmaceuticals and pesticides in reclaimed water: Efficiency assessment of a microfiltration-reverse osmosis (MF-RO) pilot plant. Journal of Hazardous Materials, 282, 165–173.

60. Ruiying G.,Jianlong W.2007. Effects of pH and temperature on isotherm parameters of chlorophenols biosorption to anaerobic granular sludge Gao. Journal of Hazardous Materials, 145, 398–403. Effects.

61. Padmavathy K.S..,Madhub G., Haseena P.V. 2016. A study on effects of pH, adsorbent dosage, time, initial concentration and adsorption isotherm study for the removal of hexavalent chromium (Cr (VI)) from wastewater by magnetite nanoparticles. Procedia Technology, 24, 585–594.

62. Santaeufemia S.,Torres E.,Abalde R.M., Abalde J. 2016. Bioremediation of oxytetracycline in seawater by living and dead biomass of the microalga Phaeodactylum tricornutum. Journal of Hazardous Materials, 320, 315–325.

63. Sulaymon A.H.,Ebrahim S.E.,Ridha M.J.M. 2012. Comparative Biosorption Of Pb(II), Cr(III) AND Cd(II) Ions In Single Component System By Live And Dead Anaerobic Biomass, Bath Study. Journal of Engineering, 18, 710–716.

64. Suresh Kumar K.,Dahms H.U.,Won E.J.,Lee J.S.,Shin K.H. 2015. Microalgae - A promising tool for heavy metal remediation. Ecotoxicology and Environmental Safety.

65. Thung I.,Aramin H.,Vavinskaya V.,Gupta S.,Park J.Y.,Crowe S.E.,Valasek M.A. 2016. Review article: The global emergence of Helicobacter pylori antibiotic resistance. Alimentary Pharmacology and Therapeutics.

66. Tokuşoglu O., Ünal M.K. 2003. Biomass Nutrient Profiles of Three Microalgae: Spirulina platensis, Chlorella vulgaris, and Isochrisis galbana. Journal of Food Science.

67. Kolpin W.D.,Furlong E.T.,Meyer M.T.,Thurman E.M.,Zaugg S.D.,Barber L.B.,Buxton H.T.2002. Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999-2000: A National Reconnaissance. Environ. Sci. Technol., 36, 1202–1211.

68. Wang W.,Li X.,Yuan S.,Sun J.,Zheng S. 2016. Effect of resin charged functional group, porosity, and chemical matrix on the long-term pharmaceutical removal mechanism by conventional ion exchange resins. Chemosphere, 160, 71–79.

69. Xie Y.,Li H.,Wang X.,Ng I.S.,Lu Y.,Jing K. 2014. Kinetic simulating of Cr(VI) removal by the waste Chlorella vulgaris biomass. Journal of the Taiwan Institute of Chemical Engineers.

70. Xiong J.Q., Kim S.J., Kurade M.B., Govindwar S.,Abou-Shanab R.A.I., Kim J.R., Roh H.S., Khan M.A., Byong-Hun Jeon B.H.2019. Combined effects of sulfamethazine and sulfamethoxazole on a freshwater microalga, Scenedesmus obliquus: toxicity, biodegradation, and metabolic fate. Journal of Hazardous Materials., 370, 138–146.

71. Qiang X.J.,Kurade M.B.,Hun J.B. 2016. Biodegradation of levofloxacin by an acclimated freshwater microalga, Chlorella vulgaris. Chemical Engineering Journal.

72. Yadav A.K.,Abbassi R.,Gupta A.,Dadashzadeh M. 2013. Removal of fluoride from aqueous solution and groundwater by wheat straw, Sawdust and activated bagasse carbon of sugarcane. Ecological Engineering, 52, 211–218.

73. Li Y.Z., Guo L.Y., Fei T.X.,Bo L.S.,Ming Z.G.,Hua J.L.,Fang L.M.,Zhou Z.,Liu S.,Xi, C.X. 2017. Immobilization of aqueous and sediment-sorbed ciprofloxacin by stabilized Fe-Mn binary oxide nanoparticles: Influencing factors and reaction mechanisms. Chemical Engineering Journal, 314, 612–621.

74. Yang C.,Wang J.,Lei M.,Xie G.,Zeng G.,Luo S. 2010. Biosorption of zinc(II) from aqueous solution by dried activated sludge. Journal of Environmental Sciences.

75. Yuan P.,Liu D.,Fan M.,Yang D.,Zhu R.,Ge F.,Zhu J.X.,He H. 2010. Removal of hexavalent chromium [Cr(VI)] from aqueous solutions by the diatomite-supported/unsupported magnetite nanoparticles. Journal of Hazardous Materials.

76. Zhang C.L.,Qiao G.L.,Zhao F.,Wang Y. 2011. Thermodynamic and kinetic parameters of ciprofloxacin adsorption onto modified coal fly ash from aqueous solution. Journal of Molecular Liquids, 163, 53–56.

77. Zhang X.,Yan S.,Chen J.,Tyagi R.D.,Li J. 2020. Physical, chemical, and biological impact (hazard) of hospital wastewater on environment: presence of pharmaceuticals, pathogens, and antibiotic-resistance genes Xiaolei. Current Developments in Biotechnology and Bioengineering., 79–102.

78. Zheng H.,Gao Z.,Yin F.,Ji X.,Huang H. 2012. Lipid production of Chlorella vulgaris from lipid-extracted microalgal biomass residues through two-step enzymatic hydrolysis. Bioresource Technology.