Water Air Soil Pollut (2018) 229: 215
https://doi.org/10.1007/s11270-018-3859-1

Optimization and Characterization of *Cladophora* sp. Alga Immobilized in Alginate Beads and Silica Gel for the Biosorption of Mercury from Aqueous Solutions

Joy G. Mokone · Hlanganani Tutu · Luke Chimuka · Ewa M. Cukrowska

Received: 9 February 2018 / Accepted: 1 June 2018 / Published online: 16 June 2018

Abstract Biosorption has gained much ground as a wastewater treatment technology. In this work, modified algal biosorbents were synthesized by immobilizing *Cladophora* sp. alga in alginate beads and silica gel. The resultant biosorbents were evaluated for the retrieval of mercury from aqueous solutions using batch scale experiments. Optimal metal removal was achieved at pH 5, agitation time 60 min, initial metal concentration 100 mg L^{-1}, and temperature 16 °C. Moreover, the experimental data fitted the Langmuir isotherm, pseudo-second-order kinetic model and Dubinin-Radushkevich isotherm thus showing that biosorption occurred on a homogeneous layer and ion exchange was the dominant mechanism. Both biosorbents also had high selectivity for Hg^{2+} in multi-elemental solutions. This work showed the potential of *Cladophora* sp. immobilized in alginate beads and silica gel in removing mercury from industrial wastewaters.

Keywords Mercury · Biosorption · *Cladophora* sp. · Alginate · Silica gel

J. G. Mokone · H. Tutu · L. Chimuka · E. M. Cukrowska (✉)
Molecular Sciences Institute, School of Chemistry, University of the Witwatersrand, Private Bag X3, Wits, Johannesburg, Gauteng, South Africa
e-mail: ewa.cukrowska@wits.ac.za

1 Introduction

Mercury is one of the most studied trace elements because it is toxic, persistent, and easily dispersed over global distances (Rezaee et al. 2006; Zeroual et al. 2003). It also exists in a variety of species, of which the organic species (methylmercury) are the most lethal due to its propensity to bioaccumulate and biomagnify in food webs thus causing severe human health complications (Han et al. 2014; Shabudeen et al. 2013). Consequently, the World Health Organization (WHO) has fixed maximum permissible levels for mercury in drinking water and industrial discharges at 1 and 5 μg L^{-1}, respectively (Mohan et al. 2001; Wang et al. 2004). Accordingly, much research work has focused on developing technologies for the abatement of mercury in industrial wastewaters to meet these stringent requirements (Oehmen et al. 2014; Urgun-Demirtas et al. 2013).

Traditionally, the methods used for the treatment of mercury-impacted wastewaters include chemical precipitation, ion exchange, membrane filtration, and coagulation (Carro et al. 2011; Figueira et al. 2011; Lohani et al. 2008; Rezaee et al. 2006). Nonetheless, some of these techniques are plagued by drawbacks such as inefficiency at low metal concentrations, high capital and operational costs, and the production of toxic secondary products (Shabudeen et al. 2013; Vasudevan et al. 2012). Hence, biosorption using biological materials is emerging as an economic, effective, and eco-friendly alternative technique (Abdel-Aty et al. 2013; Meitei and Prasad 2013; Rocha et al. 2014).

Among all the biosorbents studied, algae are the most preferred because of their wide abundance, high tolerance to metal pollutants, high surface area to volume ratio, and selectivity towards specific metals (Esmaeili et al. 2015; Gupta et al. 2010; Zeraatkar et al. 2016). Algae also have high adsorption capacities due to the presence of several functional groups like amino, amide, sulfhydryl, carboxyl, carbonyl, and hydroxyl on their cell surfaces which act as metal ligands (Amin et al. 2016; Anastopoulos and Kyzas 2015; Kumar and Oommen 2012). However, the major downside of using algae for metal biosorption applications is their fragility and tendency to disintegrate thus making it difficult to separate them from the bulk solution after completion of the process (Abu Al-Rub et al. 2004; De-Bashan and Bashan 2010; Moreno-Garrido 2008). Algal biomass also tends to clog columns during continuous flow operations thus interfering with metal removal (Ruiz-Marin et al. 2010; Sud et al. 2008). As a result, several authors recommend the immobilization of alga onto solid support matrices (De-Bashan and Bashan 2010; Kumar et al. 2016; Moreno-Garrido 2008).

The support materials that are commonly used for the immobilization of algae are alginates and silica gel (Akar et al. 2009; Cataldo et al. 2016; Muzarabani et al. 2015; Suharso et al. 2010; Petrovic and Simonic 2016; Wang et al. 2016). Alginates are biopolymers that are comprised of β-D-mannuronic and α-L-guluronic acids joined by 1–4 linkages (Bayramoğlu et al. 2006; Cataldo et al. 2013). They easily react with divalent cations like Ca^{2+} to form strong gels that can retain algae for long periods (Barquilha et al. 2017). Alginates are the immobilization agents of choice because they are non-toxic, easy to prepare and have abundant carboxyl groups which provide additional metal binding sites thus improving the biosorption capacities of the entrapped algae (Al-Rub et al. 2004). On the other hand, silica gel is a synthetic, non-toxic and inert material synthesized primarily through the reduction in pH of alkali silicate solutions to values below 10 (Suharso et al. 2010). It has a large surface area with many reactive sites which allow for the immobilization of large quantities of algal biosorbent (Akar et al. 2009; Suharso et al. 2010).

This study was aimed at evaluating the feasibility of utilizing *Cladophora* sp. alga immobilized in alginate beads and silica gel for the biosorption of mercuric (Hg^{2+}) ions from aqueous solutions. *Cladophora* sp. is a green, filamentous alga found growing in freshwaters in most areas across the globe (Barquilha et al. 2017; Lee and Chang 2011). However, very few research works report its use for the biosorption of metals (Bağda et al. 2017; Jafari and Senobari 2012; Lee and Chang 2011). In this work, the biosorption capabilities of *Cladophora* sp. alga immobilized in alginate beads and in silica gel were compared and the effects of parameters affecting Hg^{2+} biosorption were assessed. The experimental data were fit into kinetic and adsorption isotherm models. The selectivity of the biosorbents was investigated and their potential for reuse was evaluated.

2 Materials and Methods

2.1 Chemicals and Reagents

All the chemicals and reagents used in this study were of analytical grade from Sigma Aldrich, South Africa.

2.2 Preparation of Solutions

Stock solutions (1000 mg L^{-1}) were prepared by dissolving the required amounts of the nitrate salts of Hg^{2+}, Pb^{2+}, Cu^{2+}, Fe^{3+}, and Cd^{2+} in deionized water (Milli-Q, 18.2 MΩ.cm at 25 °C) purified using a Millipore Direct UV-3 (France) water purification system. Subsequently, working solutions of desired concentration were prepared by serial dilution of the stock solutions.

2.3 Preparation of the Biosorbents

Cladophora sp. alga was collected from Alexandra Dam, Springs, Johannesburg, Gauteng, South Africa. The algal samples were thoroughly washed with running tap water and then rinsed thrice with deionized water. Subsequently, the algae were cultivated in Bold's acidic medium (BAM) of known composition and harvested while they were still in the stationary growth phase (Ji et al. 2012). The algae were then acclimated in deionized water for 24 h before being air-dried on a filter paper to remove excess moisture (Zeroual et al. 2003).

2.3.1 Immobilization of Cladophora sp. Alga in Alginate Beads

This was achieved using a modified version of the procedure described by Bayramoğlu et al. (2006). First, 2 g of sodium alginate was dissolved in 50 mL of deionized water with the aid of heating. The resultant solution was cooled to room temperature (21 °C) before being mixed with an algal suspension comprised of 1.0 g algae cells in 50 mL deionized water. Afterwards, the mixture was added dropwise to 50 mL of 0.1 M $CaCl_2$ while continuously stirring gently to form beads of approximately 2 mm diameter. The formed beads were left in solution for 90 min for complete gel formation. Thereafter, the beads were rinsed thoroughly using 0.85% (*w*/*v*) NaCl solution and stored at 4 °C in 5 mM $CaCl_2$ for later use.

2.3.2 Immobilization of Cladophora sp. Alga in Silica Gel

Cladophora sp. alga was immobilized in silica gel using a modified version of the method described by Akar et al. (2009). Five grams of silica gel was dissolved in 7% (*w*/*v*) aqueous solution of KOH with the aid of heating. After cooling to room temperature, the solution was mixed with an algal suspension made up of 2.5 g of alga in 100 mL of deionized water. A few drops of 20% (*v*/*v*) phosphoric acid were added to facilitate gel formation. The gel formed was dried at 60 °C for 24 h in a WiseCube Fuzzy control (Germany) incubation system. Subsequently, the gel was powdered and sieved to give particles of size 0.5 to 1.0 mm and stored in PTFE tubes for use in experiments.

2.4 Characterization of the Biosorbents

Powdered samples (particle size 0.5 to 1.0 mm) of the test biosorbents were characterized using a Bruker Tensor 27 (Germany) Fourier transform infrared spectrophotometer (FTIR) for functional group identification and an FEI Quanta 200 (USA) scanning electron microscope (SEM) for surface morphology studies. Thermogravimetric analysis also was performed using a Perkin Elmer STA 6000 (USA) thermal analyzer to deduce the thermostability of the algal-based biosorbent materials. The surface areas and pore volumes of the biosorbents were determined using the Braunner-Emmet-Taylor (BET) technique on a Micrometrics Trista 3000 (USA) analyzer.

2.5 Batch Biosorption Tests

Biosorption studies were conducted in 100 mL Erlenmeyer flasks containing 0.5 g (dry weight) of biosorbent suspended in 50 mL of metal solution of known concentration held at pH 5. The reaction vessels were agitated on a Labcon 3100E (USA) rotary shaker for prescribed time periods while keeping the temperature constant at 25 °C. After agitation, the test biosorbents were separated from solution via gravimetric filtration and the concentration of mercury remaining in the filtrates was measured. The biosorption capacities (q) were determined using Eq. 1 (Lee and Chang 2011; Tuzen et al. 2009).

$$q = (C_0 - C_e) \times V/M \quad (1)$$

where q is the adsorption capacity (mg g^{-1}), C_0 and C_e are the initial and equilibrium metal concentrations (mg L^{-1}), V is the volume of solution (L), and M is the mass of sorbent (g).

The effects of pH (3–8.5), initial metal concentration (1–100 mg L^{-1}), contact time (0–120 min), and temperature (16–40 °C) on the biosorption capacities were also investigated. Furthermore, the elemental speciation of the solutions was studied using the PHREEQC geochemical modelling code (Parkhurst and Appelo 2013).

2.6 Effect of Competing Ions

The effect of the presence of competing cations on the biosorption of Hg^{2+} by *Cladophora* sp. alga immobilized in alginate beads and silica gel was evaluated by conducting biosorption in the manner described in Section 2.5 except that each biosorbent was suspended in multi-elemental solution containing 1 mg L^{-1} each of Hg^{2+}, Pb^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, and Cd^{2+} ions. The selectivity of the biosorbents was evaluated in terms of the adsorption capacity and distribution co-efficient (K_D). K_D was determined using the following equation (Lee and Chang 2011; Tuzen et al. 2009).

$$K_D = \left(C_0 - C_f / C_f\right) \quad (2)$$

where K_D is the distribution co-efficient and C_0 and C_f are the concentrations before and after exposure to the biosorbents.

2.7 Reusability Studies

Mercury-loaded biosorbents were regenerated by placing them in 50 mL beakers containing 10 mL of 0.1 M HCl and shaking continuously for 2 h at 200 rpm. The biosorbents were then separated from the desorption medium and washed thoroughly with deionized water before being reused for biosorption. This process was repeated for three successive adsorption-desorption cycles and the efficiencies of *Cladophora* sp. alga immobilized in alginate beads and silica gel were determined after each cycle.

2.8 Metal Analysis

All the solutions were filtered using 0.45 μM membrane filters prior to analysis. Thereafter, their mercury content was measured using a Perkin Elmer FIMS 400 (USA) mercury analyzer. On the other hand, an inductively coupled plasma optical emission spectroscopy (ICP-OES), Spectro Genesis FEE (Germany) was used for the determination of the concentrations of Pb^{2+}, Cu^{2+}, Cd^{2+}, and Fe^{3+} in multi-metal solutions before and after metal binding. The accuracy of the method was validated using a certified reference material (BCR 482 for trace elements in lichens) and elemental standards were used throughout the analyses.

3 Results and Discussion

3.1 Functional Group Identification

The FTIR spectra of *Cladophora* sp. immobilized in alginate beads and silica gel before and after mercury binding are illustrated in Fig. 1. Prior to metal biosorption, the spectrum for the alga immobilized in alginate beads had four prominent peaks corresponding to the overlapping of the –OH and –NH stretching (3408 cm^{-1}), asymmetric and symmetric stretching vibration of the –C=O in the carboxyl group (1602 and 1419 cm^{-1}) and the C–O–C– vibration stretching (1020 cm^{-1}) (Petrovic and Simonic 2016). These peaks are characteristic of the functional groups from both the alga and alginate support matrix thus verifying that immobilization had successfully been performed.

However, mercury binding onto the biosorbent resulted in the suppression of the peak at 3408 cm^{-1} and the depreciation in the intensities of the bands at 1606 and 1409 cm^{-1}. This suggested that the –OH, –NH, and –C=O groups were major contributors in the mechanism for the biosorption of mercury onto *Cladophora* sp. alga immobilized in alginate beads. Notwithstanding, the most significant change caused by the binding of mercury onto this biosorbent was the reappearance of the peak at 2356 cm^{-1} due to the –CH group in alginate (Daemi and Barikani 2012). This was attributable to the fact that during metal binding, mercury which has a higher affinity for nitrogen-containing groups replaced the –C–O–C– functional group which previously interacted with the amine group thus resulting in the formation of a new –CH bond (Daemi and Barikani 2012).

In the same manner, the FTIR spectrum of the alga immobilized in silica gel originally had five conspicuous peaks at 3010, 1739, 1367, 1217, and 640 cm^{-1}. The peaks were assigned to the overlapping of the –OH and –NH groups, asymmetric and symmetric stretching

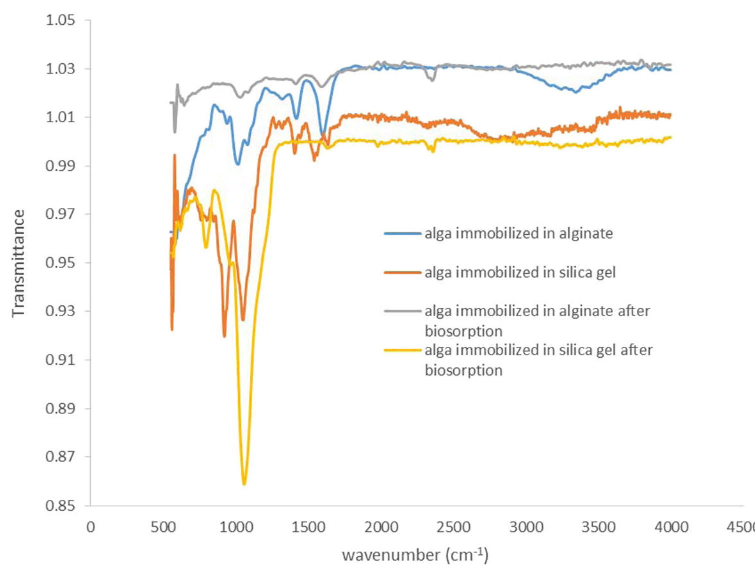

Fig. 1 FTIR spectra of *Cladophora* sp. alga immobilized in alginate beads and silica gel before and after mercury biosorption (scanning range 400–4500 cm^{-1})

vibration of the –C=O group, and –Si–O–Si and Si–O– groups, respectively (Akar et al. 2009). This showed that the alga immobilized in silica gel also had functional groups originating from both the alga and silica gel thus verifying that immobilization had occurred. Nonetheless, mercury binding resulted in the suppression of the peaks at 3010, 1739, 1367, and 1217 cm^{-1} thus implying that the –OH, –NH, Si–O–, and –C=O– participated in the process for the biosorption of mercury. Nonetheless, the most prominent indication of mercury biosorption onto the surface of *Cladophora* sp. immobilized in silica gel was the appearance of a new peak at 1066 cm^{-1}. This was attributed to the reappearance of the Si–OH group in silica gel due to loss of interaction of Si–O– with the amine group (Akar et al. 2009). This observation provided proof that the –NH group is one of the major contributors towards the biosorption of mercury from aqueous solutions by *Cladophora* sp. alga immobilized in silica gel. The results obtained for functional group identification on the surfaces of biosorbents before and after metal biosorption are congruent with those reported by others (Akar et al. 2009; Torres et al. 2005; Petrovic and Simonic 2016).

3.2 Surface Morphology Studies

Surface studies using SEM revealed significant changes in the morphologies of the biosorbents after mercury biosorption (Fig. 2). The surface morphology of the alga immobilized in alginate beads was smooth and became distorted after mercury binding. Similarly, the morphology of *Cladophora* sp. alga immobilized in silica gel was originally organized and comprised of algal filaments attached to the surface. After binding, the surface became rugged and showed an irregular distribution of particle and crystalline deposits adhered to it. These changes in morphology were attributed to exposure of the biosorbent surface to mercury.

3.3 Thermostability Studies

The results for the thermostability of *Cladophora* sp. immobilized in alginate beads and silica gel (Fig. 3) showed that the two biosorbents followed different thermal decomposition pathways. In the case of the *Cladophora* sp. immobilized in alginate beads, thermal decomposition occurred via four steps viz. moisture loss (25–240 °C), initial destruction of glycosidic bonds (240–270 °C), followed by further degradation of glycosidic bonds (270–330 °C), and gradual degradation of all previously formed carbonaceous materials (330–530 °C) (Patel et al. 2016).

On the contrary, the thermograph for the *Cladophora* sp. immobilized in silica gel was much simpler and occurred in only two steps due to the loss of physisorbed water (100–150 °C) and condensation of vicinal groups (200–600 °C) (El-Naggar and Si 2013). These results showed that the test biosorbents are stable up to high temperatures and would be suitable for their intended purpose. The observations made were similar to these

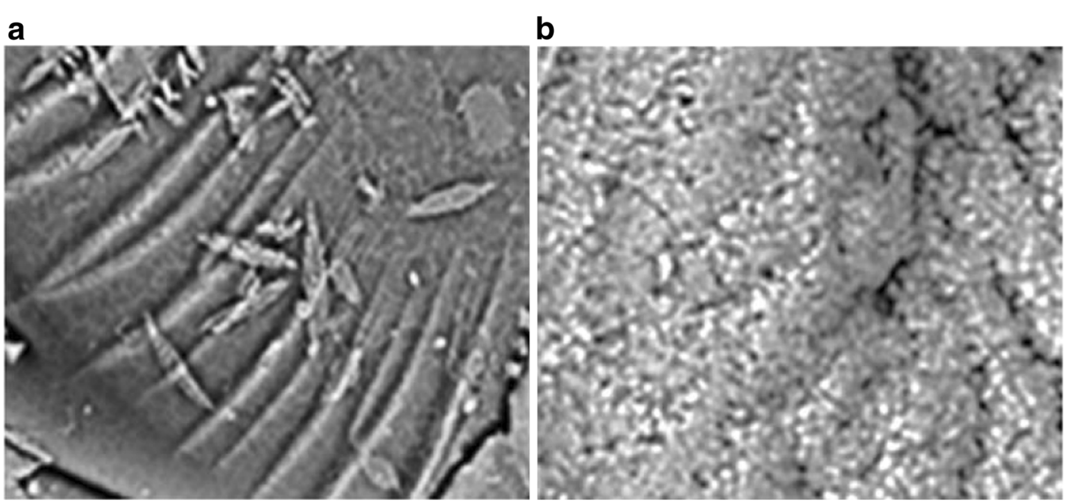

Fig. 2 SEM micrographs showing the morphology of *Cladophora* sp. alga immobilized in **a** silica gel and **b** alginate beads

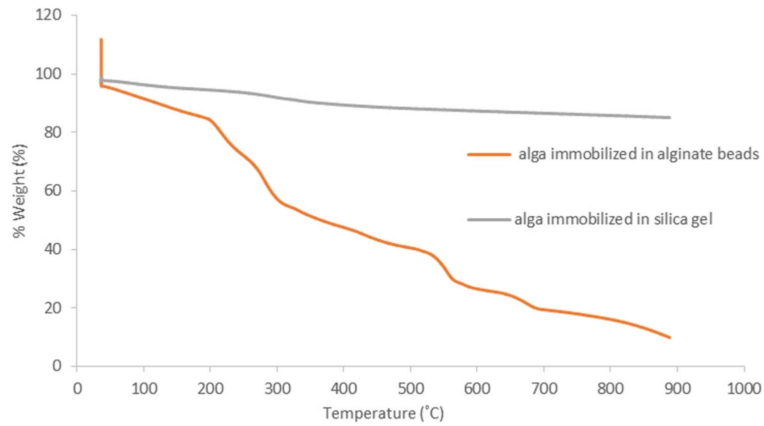

Fig. 3 TGA thermographs for *Cladophora* sp. alga immobilized in alginate beads and *Cladophora* sp. alga immobilized in silica gel (36–900 °C)

reported in the literature (El-Naggar and Si 2013; Qiusheng et al. 2015).

3.4 Surface Areas and Pore Volumes of the Biosorbents

BET analysis revealed that the surface area and pore volume of the *Cladophora* sp. alga immobilized in alginate beads were 6.234 $m^2\ g^{-1}$ and 0.02526 $cm^3\ g^{-1}$, respectively, while those of the alga immobilized in silica gel were 5.682 $m^2\ g^{-1}$ and 0.0187 $m^2\ g^{-1}$, respectively. These values compared well with other algal-based biosorbents reported in the literature (Song et al. 2013; Qiusheng et al. 2015). However, mercury binding negatively impacted the surface areas and pore volumes of both the biosorbents studied. The surface area and pore volume of the alga immobilized in alginate beads decreased to 3.961 $m^2\ g^{-1}$ and 0.01274 $cm^3\ g^{-1}$ respectively while those of the alga immobilized in silica gel dropped to 4.199 $m^2\ g^{-1}$ and 0.009421 $cm^3\ g^{-1}$, respectively. This observation was attributed to the blockage of some of the pores on the biosorbents from nitrogen passage by the bound mercury (Ahmady-Asbchin et al. 2012; Bayramoğlu et al. 2006; Gupta and Rastogi 2006).

3.5 Batch Biosorption Studies

3.5.1 Effect of pH

The effect of pH on the biosorption of mercury by *Cladophora* sp. alga immobilized in alginate beads and *Cladophora* sp. immobilized in silica gel is displayed in Fig. 4a. For both biosorbents studied, the adsorption capacities were lowest at pH 3 due to the abundance of protons which compete with metal ions for the active binding sites (Sud et al. 2008; Zeroual et al. 2003). Protonation of the sites at this pH also results in the repulsion of mercuric ions thus further reducing the adsorption capacities (Gupta and Rastogi 2008). Accordingly, increasing the pH up to 5 enhanced the biosorption.

Nonetheless, increasing the pH to 6.5 and 8.5 led to a significant reduction in the adsorption capacity of the biosorbent. This drastic decline in adsorption capacity at higher pH values is attributed to metal precipitation which lowered the concentration of free metal ions available for metal binding (Bayramoğlu et al. 2006; Singh et al. 2014). Speciation studies using the PHREEQC geochemical modelling revealed that $HgCl_2$ is the main mercury species in solution at pH 5 while the dominant specie at pH values > 6 is $Hg(OH)_2$. Therefore, the optimum pH for removal of Hg^{2+} by *Cladophora* sp. alga immobilized in alginate beads and *Cladophora* sp. immobilized in silica gel was 5 and all subsequent biosorption studies in this work were performed at this pH. These results are congruent to those obtained by Abdel-Aty et al. (2013) for the removal of Cd (II) and Pb (II) ions from aqueous solutions using *Anabaena sphaerica* biomass. They deduced that the optimum pH for metal uptake was 5.5.

It is also worth noting that for all the pH values studied, the adsorption capacity of the alga immobilized in alginate beads was higher than that of the alga entrapped in silica gel. This is because the oxygen atoms in the silanol (=Si–OH) and siloxane groups on the surface of silica gel are poor electron donors compared to those in the carboxyl group of alginates (Suharso et al. 2010).

3.5.2 Effect of Contact Time and Kinetic Modelling

The time profiles for the evaluation of the effect of agitation time on the biosorption performance of *Cladophora* sp.

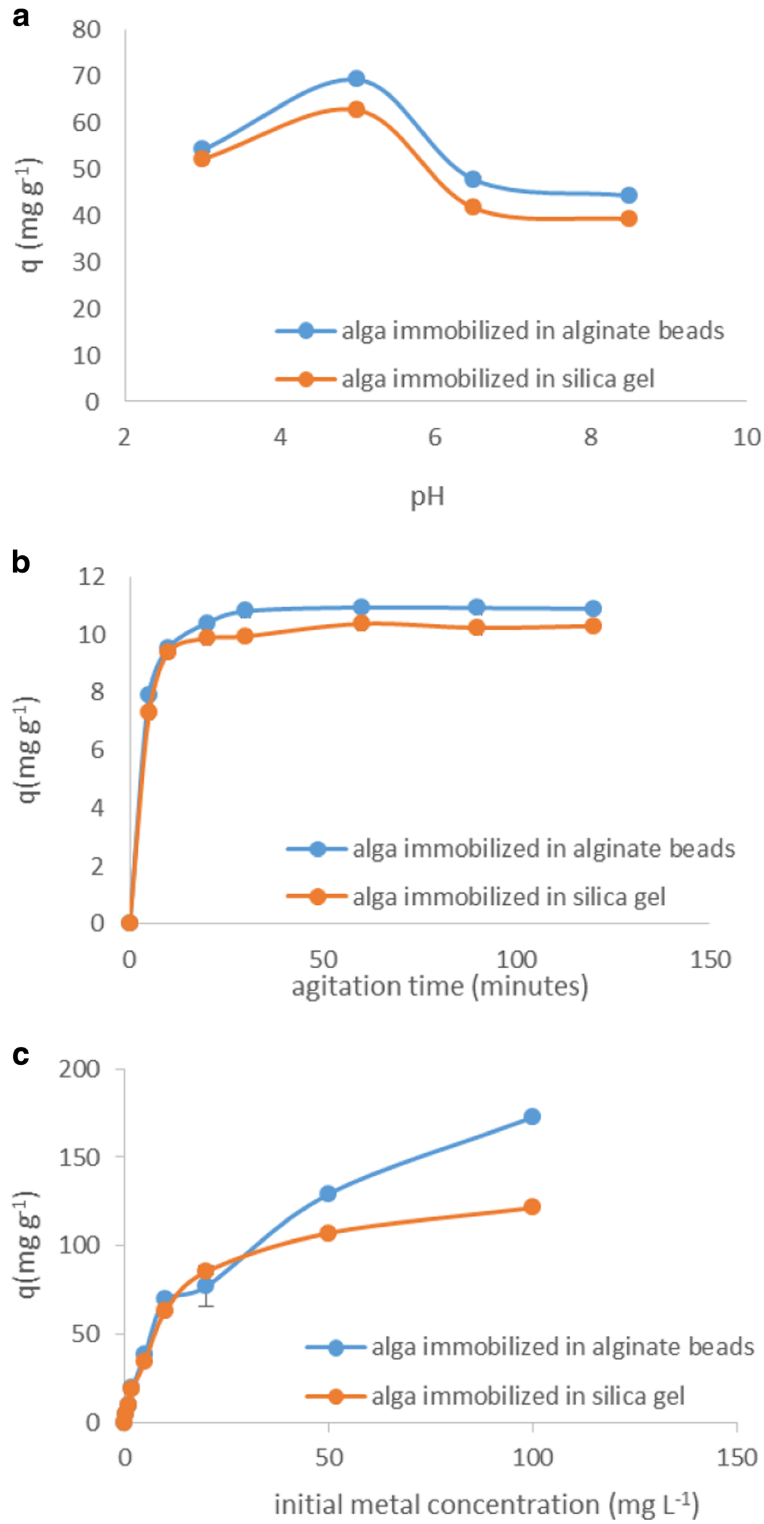

Fig. 4 Effect of **a** pH, **b** agitation, and **c** initial metal concentration on the biosorption of merucry from aqueous solutions by *Cladophora* sp. alga immobilized in alginate beads and *Cladophora* sp. alga immobilized in silica gel

alga immobilized in alginate beads and silica gel are illustrated in Fig. 4b. For both biosorbents studied, biosorption was fastest during the initial stages of the process and most of the metal ions in solution were adsorbed within 30 min.

This was due to the abundance of metal binding sites at the start of the process (Bayramoğlu et al. 2006).

The rate of adsorption capacity decreased with time due to the exhaustion of binding sites on the biosorbent surfaces (Apiratikul and Pavasant 2008). Ultimately, a plateau was reached at 60 min for both biosorbents. Therefore, increasing the agitation time beyond this point had no significant impact on the biosorption capacities of the biosorbents. Consequently, the equilibrium times for the sequestration of Hg^{2+} from aqueous solution by *Cladophora* sp. alga immobilized in alginate beads and silica gel were both set at 60 min. These results are comparable to those for metal biosorption from aqueous solutions using other algal biosorbents (Cazón et al. 2013; Jafari and Senobari 2012).

The kinetic data was modelled against the pseudo-first order, pseudo-second order, and Webber-Morris intraparticle diffusion models whose linear mathematical expressions are illustrated in Eqs. 3, 4, and 5, respectively (Apiratikul and Pavasant 2008; Tuzen et al. 2009).

$$\ln(q_e - q_t) = lnq_e^{-k_1} t \quad (3)$$

where q_t and q_e are the adsorption capacities at time t and equilibrium, respectively (mg g^{-1}), k_1 is the rate constant for pseudo-first-order kinetics.

The values of k_1 and q_e were obtained from the slope and intercept of the linear plot of $\ln(q_e - q_t)$ versus t.

$$t/q_t = 1/k_2 q_e^2 + 1/q_e t \quad (4)$$

where k_2 is the rate constant (g mg^{-1} min^{-1}), q_t is the biosorption capacity at time t, q_e is the biosorption capacity at equilibrium, and the values of k_2 and q_e were deduced from the slope and intercept of the linear plot of $\frac{t}{q_t}$ against t.

$$q_t = k_1 \sqrt{t} \quad (5)$$

where k_1 is the intraparticle diffusion rate constant (mg g^{-1} $min^{-0.5}$), q_t is amount metal adsorbed (mg g^{-1}), t is the time in minutes, and k_1 was determined from the slope of q_t versus t.

Table 1 gives a summary of the results for the kinetic modelling of the biosorption data. For both biosorbents, the values of the correlation ratio (R^2) for the pseudo-second-order model were closer to unity than those for the pseudo-first-order model. The calculated values of the adsorption capacity of the biosorbents at equilibrium ($q_{e,\ calc}$) for the pseudo-second-order model were also closer to the experimental values than those determined using the pseudo-second-order model. This signified that the kinetics for the biosorption of Hg^{2+} onto *Cladophora* sp. alga immobilized in alginate beads and silica gel were best described by the pseudo-second-order model and chemisorption was the rate limiting mechanism (Kumar et al. 2016; Tuzen et al. 2009). These findings are aligned to those reported by other researchers for the kinetics studies for the biosorption of metal ions using different algae and algal-based biosorbents (Abu Al-Rub et al. 2004; Apiratikul and Pavasant 2008; Tuzen et al. 2009).

The values of the pseudo-first order and pseudo-second-order rate constants (k_1 and k_2) calculated for *Cladophora* sp. alga immobilized in silica gel were higher than those determined for *Cladophora* sp. alga immobilized in alginate beads. This is mainly because there are larger mass transfer and diffusion limits in the latter as the alga is completely entrapped in the beads (Al-Rub et al. 2004). Nonetheless, these effects were counter-balanced by the abundance of carboxyl groups in the alga immobilized in alginate beads which substantially enhanced the biosorbent's adsorption capability (Bayramoğlu et al. 2006; Suharso et al. 2010).

On the other hand, the findings for the Webber-Morris model demonstrated that the biosorption of Hg^{2+} by *Cladophora* sp. immobilized in alginate beads and silica gel was limited by diffusion only at the start of the process. This is mainly due to the fact that during this phase, diffusion plays a key role in transporting metal ions towards the biosorbent surfaces (Apiratikul and Pavasant 2008). Hence, Eq. 4 was only applied to the initial stages of the biosorption process and the R^2 values obtained for this model were 0.9958 for the alga immobilized in alginate beads and 0.9961 for the alga immobilized in silica gel. In addition, the values of the intraparticle diffusion constant (k_1) showed that the rate of diffusion was faster for the biosorption system using the alga immobilized in silica gel than when using the alga immobilized in alginate beads. This is mainly because in the latter form, *Cladophora* sp. is completely engulfed within the beads; therefore, it experiences the highest mass transfer resistance. However, this effect became less significant as more time elapsed and other mechanisms took precedence.

3.5.3 Effect of Initial Metal Concentration and Adsorption Isotherm Modelling

The relation between the initial metal concentration in solution and the biosorption capacities of *Cladophora*

Table 1 Summary of the results for the kinetic modelling of the biosorption of Hg^{2+} using *Cladophora* sp. alga immobilized in alginate beads and silica gel (pH 5, initial metal concentration 1 mg L^{-1}, biosorbent dosage 10 mg L^{-1}, temperature 25 °C)

Biosorbent	q_e	Pseudo-first order				Pseudo-second order			Webber-Morris	
		R^2	$q_{e,\ calc}$	k_1	b	R^2	$q_{e,\ calc}$	k_2	R^2	k_1
Cladophora sp. alga immobilized in alginate beads	19.84	0.7186	2.832	0.02789	0.4804	0.9983	20.83	10.08	0.9958	2.152
Cladophora sp. alga immobilizedin silica gel	17.60	0.5435	2.229	0.03379	0.3694	0.9989	18.04	10.21	0.9961	2. 305

sp. alga immobilized in alginate beads silica gel is illustrated on the Fig. 4c. It is evident that increasing the initial metal concentration enhanced the biosorption capacities of both the biosorbents studied. This is attributable to the increased probability of interaction between the metal ions and binding sites at higher metal concentrations (Kumar and Oommen 2012). This trend was apparent up to an initial metal concentration of 100 mg L^{-1}; hence, the optimum concentration for maximal mercury biosorption was set as 100 mg L^{-1} for both biosorbents. Moreover, the maximum adsorption capacities of the biosorbents were 121.3 and 172.5 mg g^{-1} for *Cladophora* sp. alga immobilized in silica gel and *Cladophora* sp. alga immobilized in alginate beads, respectively. These findings are in agreement to those published by other researchers for the uptake of various metal ions using different unmodified and immobilized algal biosorbents (Barquilha et al. 2017; Bayramoğlu et al. 2006; Muzarabani et al. 2015).

Adsorption isotherm modelling of the equilibrium data was also performed using the Langmuir, Freundlich, and Dubinin-Radushkevich isotherms whose linear versions are given in Eqs. 6, 7, and 8 (Tuzen et al. 2009).

$$C_e/q_e = 1/b.q_m + C_e/q_m \tag{6}$$

where C_e is the concentration of metal ions in solution at equilibrium, q_e is the adsorption capacity of the sorbent at equilibrium, q_m is maximum adsorption capacity, and b is the Langmuir constant related to suitability of sorbent-sorbate system. The values of q_m and b were deduced from the slope of the linear plot of C_e/q_e versus C_e.

$$logq_e = logK_F + 1/n.logC_e \tag{7}$$

where K_F is the Freundlich constant indicative of the adsorption capacity and n is the adsorption intensity. The values of n and K_F were determined from the slope and intercept of the linear graph of log q_e against log C_e

$$ln\ q_e = lnq_m - \beta\varepsilon^2 \tag{8}$$

where β is the activity co-efficient related to energy and ε is the Polanyi potential given by $RT[\ln(1 + 1/C_e)]$.

The values of β and q_m were calculated from the slope and intercept of the linear plot of ln q_e versus ε^2 and the sorption energy for the biosorption process was calculated using Eq. 8 (Dada et al. 2012).

$$E_S = 1/\left(\sqrt{2\beta}\right) \tag{9}$$

The adsorption isotherm modelling results (Table 2) revealed that the R^2 values for the Langmuir isotherm were 0.9425 and 0.9926 for the alga immobilized in alginate beads and alga immobilized in silica gel, respectively. On the contrary, those for the Freundlich isotherm were 0.9194 and 0.9088 for the same biosorbent thus indicating that the R^2 values for the Langmuir isotherm were closest to unity. Furthermore, the maximum adsorption capacities for the test biosorbents calculated using the Langmuir isotherm were closer to the experimental values than those calculated using the Freundlich isotherm. This indicated that the biosorption of mercury onto *Cladophora* sp. alga immobilized in alginate beads and silica gel occurred on a homogeneous layer with actives sites of equivalent energies (Khoramzadeh et al. 2013; Tuzen et al. 2009).

The values of the affinity constant (b) calculated using the Langmuir isotherm were 0.4807 and 0.3694 for *Cladophora* sp. alga immobilized in alginate beads and *Cladophora* sp. alga immobilized in silica gel, respectively. This showed that *Cladophora* sp. alga immobilized in alginate beads had a higher affinity for Hg^{2+} ions than *Cladophora* sp. alga immobilized in silica gel thus agreeing with the observations that *Cladophora* sp. alga immobilized in alginate beads had the highest metal uptake capacity. Moreover, the values of the adsorption intensity (n) determined using the Freundlich isotherm were 2.8161 and 2.5833 for the *Cladophora* sp. alga immobilized in alginate beads and *Cladophora* sp. alga immobilized in silica gel,

Table 2 Freundlich, Langmuir, and Dubinin-Radushkevich isotherm modelling parameters for the biosorption of Hg^{2+} using *Cladophora* sp. alga immobilized in alginate beads and silica gel (temperature 25 °C, biosorbent dosage 10 g L^{-1}, pH 5, agitation time 60 min)

Biosorbent	Freundlich isotherm			Langmuir isotherm			Dubinin-Radushkevich isotherm		
	R^2	K_F	n	R^2	q_m	b	R^2	X_m	E_s
Cladophora sp alga immobilized in alginate beads	0.9194	33.11	2.816	0.9425	172.4	0.4807	0.9133	158.7	9.406
Cladophora sp. alga immobilized in silica gel	0.9088	30.41	2.583	0.9926	121.9	0.3694	0.9034	106.4	9.691

respectively. This provides further proof that biosorption is more favorable in the *Cladophora* sp. alga immobilized in alginate beads.

The equilibrium data also fitted the Dubinin-Radushkevich isotherm reasonably well with R^2 values of 0.9133 and 0.9034 for the *Cladophora* sp. immobilized in alginate beads and *Cladophora* sp. immobilized in silica gel, respectively. Moreover, the sorption energies (E_s) for the biosorbents were 9.406 and 9.690 kJ mol^{-1} for the *Cladophora* sp. immobilized in alginate beads and *Cladophora* sp. immobilized in silica gel, respectively. This suggested that the governing mechanism for the biosorption of Hg^{2+} by *Cladophora* sp. alga immobilized in alginate beads and *Cladophora* sp. immobilized in silica gel was ion exchange. This correlated with the findings for the kinetic modelling which revealed that the rate limiting mechanism was chemisorptive in nature.

The results obtained for the adsorption isotherm modelling of equilibrium data for the biosorption of mercury by *Cladophora* sp. alga immobilized in alginate beads compared well with those reported by Sheikha et al. (2008) for the biosorption of Zn^{2+} ions onto blank alginate beads and *Chlorella pyenoidosa* alga immobilized in alginate beads.

3.5.4 Effect of Temperature and Thermodynamic Modelling

The effect of temperature on the biosorption capacities of *Cladophora* sp. immobilized in alginate beads and *Cladophora* sp. immobilized in silica gel was investigated and the Gibbs free energy at different temperatures (ΔG), enthalpy (ΔH), and entropy (ΔS) of the biosorption process were calculated using Eqs. 10 and 11 (Tuzen et al. 2009).

$$\Delta G^{\circ} = -RTlnK_D \tag{10}$$

where $\Delta G°$ is the Gibbs free energy (kJ mol^{-1}), R is the universal gas constant (8.314 J mol^{-1} K^{-1}), T is the temperature in K and $K_D = C_e/q_e$

$$ln\ K_D = \Delta S^{\circ}/R - \Delta H^{\circ}/RT \tag{11}$$

where ΔH (enthalpy) and ΔS (entropy) are determined from the slope and intercept of the Vant Hoff plot of ln K_D versus 1/T.

It was inferred that the adsorption capacities of *Cladophora* sp. alga immobilized in alginate beads were 43.87, 41.42, 29.83, and 17.43 mg g^{-1} for 16, 25, 30, and 40 °C respectively while those for *Cladophora* sp. alga immobilized in silica gel were 39.47, 37.37, 25.44, and 17.43 mg g^{-1} for the same temperatures. This showed that the biosorption capacities of both biosorbents studied depreciated when the temperature increased thus indicating that the biosorption processes were exothermic. Moreover, the ΔG values for the alga immobilized in alginate beads were − 10.54, − 4.851, − 3.642, and − 0.8633 kJ mol^{-1} for 16, 25, 30, and 40 °C respectively while those of the alga immobilized in silica gel were − 9.897, − 4.802, − 2.903, and − 0.5672 kJ mol^{-1} respectively for the same temperatures. This observation also showed that for both biosorbents studied, the value of ΔG became less negative thus signifying a decrease in the spontaneity of the processes with increases in temperature.

The enthalpies of the biosorption process were − 86.96 and − 73.60 kJ mol^{-1} for the alga immobilized in alginate beads and also immobilized in silica gel. All the above observations showed that the biosorption of Hg^{2+} by both test biosorbents was exothermic in nature and favored at low temperature. However, the values of adsorption capacity, ΔG and ΔH for the alga immobilized in silica gel were lower than those of the alga immobilized in alginate beads thus further proving that the latter had a higher affinity for Hg^{2+} ions. Regardless, the entropies of the biosorption process using the alga immobilized in alginate were 0.2635 kJ mol^{-1} while that of the alga immobilized in silica gel was 0.2214 kJ mol^{-1} thus indicating that the randomness at the solid-liquid boundary reduced after the biosorption process (Tuzen et al. 2009).

3.5.5 Effect of Competing Ions

The results for the effect of the presence of competing ions on the biosorption of mercuric ions by *Cladophora* sp. alga immobilized in alginate beads and *Cladophora* sp. alga immobilized in silica gel revealed that the adsorption capacities of both biosorbents declined in multi-metal solutions. This was mainly due to the competition among the metal ions for the finite number of binding sites available on the biosorbents (Singh et al. 2007). However, the largest reduction in q_e was observed in the case of the alga immobilized in silica gel whereby the value of q_e dropped from 9.756 to 5.841 mg g^{-1} (40%). On the other hand, the biosorption performance of the alga immobilized in alginate beads dropped from 9.812 to 6.855 mg g^{-1} (30%).

A comparison of the K_D values also showed that the alga immobilized in silica gel was most impacted by the presence of competing ions. The K_D of the biosorbent dropped from 86.88 L g^{-1} in the unitary solution to 14.88 L g^{-1} in the multi-metal solution (83%). On the other hand, the K_D value for the alga immobilized in alginate beads decreased from 89.70 to 20.01 L g^{-1} (78%). This provided further evidence that *Cladophora* sp. immobilized in alginate beads had a higher selectivity for mercury in multi-elemental solutions. It was also observed that the affinities of the test biosorbents for the metals in solution followed the order $Hg^{2+} > Pb^{2+} > Cu^{2+} > Fe^{3+} > Co^{2+} > Cd^{2+}$. The differences in affinities of the metal ions were attributed to varying ionic radii, electronegativities, and redox potentials (Dönmez et al. 1999). Moreover, according to Pearson's hard and soft acid base (HSAB) theory, Cd, Co are soft metals which will bind only to soft ligands like amine. On the contrary, Hg and Pb are able to bind to a variety of the functional groups on the biosorbents (Mishra et al. 2016).

This biosorptive behavior was similar to that described by Singh et al. (2007) for the removal of Cu^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+}, and Zn^{2+} from multi-metal solutions using *Spirogyra neglecta, Pithophora oedogonia, Cladophora calliceima, Hydrodictyon reticulatum,* and *Aulosira fertilissima* algae. They observed that the metal uptake capabilities of the algae decreased in multi-metal solutions and the biosorbent affinities for the test metal ions followed the order Ni < Zn < Cd < Cu < Pb (Singh et al. 2007).

3.5.6 Reusability Studies

The results for the recyclability of the test biosorbents are presented in Fig. 5 and they demonstrated that the biosorption efficiency of *Cladophora* sp. alga immobilized in alginate beads was enhanced after completion of the first cycle. This was attributed to the fact that the acidic medium was able to remove the contaminants adhered to the binding sites on the biosorbent thus freeing them for mercury binding (Abu Al-Rub et al. 2004). Nonetheless, henceforth, there was a gradual reduction in the efficiency of the biosorbent with increases in the number of adsorption-desorption cycles. In fact, *Cladophora* sp. alga immobilized in alginate beads retained efficiency greater than 80% even after three successive cycles. However, a different trend was observed for the alga immobilized in silica gel. The removal efficiency of the biosorbent decreased to

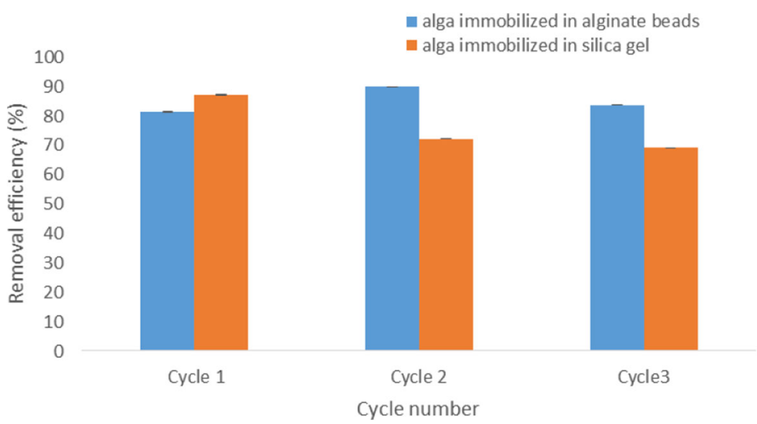

Fig. 5 Reusability of *Cladophora* sp. alga immobilized in alginate beads and *Cladophora* sp. alga immobilized in silica gel for the biosorption of merucry from aqueous solutions

87.11% after the first cycle and 72.26% in the second cycle before finally dropping to 69.15% in the third cycle. These observations show that both biosorbents could be reused for up to three successive cycles without substantial loss in removal efficiency. However, the alga immobilized in alginate beads could be used for longer retaining over 80% of its biosorption capacity.

4 Conclusion

This study revealed that *Cladophora* sp. alga immobilized in both alginate beads and silica gel biosorbents had a reasonable potential for removing Hg^{2+} from aqueous solutions. However, *Cladophora* sp. alga immobilized in alginate beads had a higher biosorption capacity and selectivity towards Hg^{2+} than *Cladophora* sp. alga immobilized in silica gel. Based on these observations, *Cladophora* sp. alga immobilized in alginate beads has the greatest potential for use in wastewater treatment applications. Nonetheless, there is still a need to investigate the industrial applicability of the biosorbent using continuous flow studies.

Acknowledgments The authors are thankful to the Government of Botswana and the PMA at the University of the Witwatersrand for financial support for Joy G. Mokone.

FundingThis research did not receive any specific grant from funding agencies in public, commercial, or non-profit sectors.

References

Abdel-Aty, A. M., Ammar, N. S., Ghafar, H. H. A., & Ali, R. K. (2013). Biosorption of cadmium and lead from aqueous solution by fresh water alga *Anabaena sphaerica* biomass. *Journal of Advanced Research, 4*(4), 367–374.

Abu Al-Rub, F. A., El-Naas, M. H., Benyahia, F., & Ashour, I. (2004). Biosorption of nickel on blank alginate beads, free and immobilized algal cells. *Process Biochemistry, 39*(11), 1767–1773.

Ahmady-Asbchin, S., & Azhdehakoshpour, A. (2012). Biosorption of Cu (II) and Ni (II) ions from aqueous solution by marine brown algae Sargassum angustifolium. *Journal of Biodiversity and Environmental Sciences, 6*(18), 271–279.

Akar, T., Kaynak, Z., Ulusoy, S., Yuvaci, D., Ozsari, G., & Akar, S. T. (2009). Enhanced biosorption of nickel (II) ions by silica-gel-immobilized waste biomass: biosorption characteristics in batch and dynamic flow mode. *Journal of Hazardous Materials, 163*(2), 1134–1141.

Amin, F., Talpur, F. N., Balouch, A., Chandio, Z. A., Surhio, M. A., & Afridi, H. I. (2016). Biosorption of mercury (II) from aqueous solution by fungal biomass Pleurotus eryngii: isotherm, kinetic, and thermodynamic studies. *Environmental Progress & Sustainable Energy, 35*(5), 1274–1282.

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

Apiratikul, R., & Pavasant, P. (2008). Batch and column studies of biosorption of heavy metals by *Caulerpa lentillifera*. *Bioresource Technology, 99*(8), 2766–2777.

Bağda, E., Tuzen, M., & Sarı, A. (2017). Equilibrium, thermodynamic and kinetic investigations for biosorption of uranium with green algae (Cladophora hutchinsiae). *Journal of Environmental Radioactivity, 175*, 7–14.

Barquilha, C. E. R., Cossich, E. S., Tavares, C. R. G., & Silva, E. A. (2017). Biosorption of nickel (II) and copper (II) ions in batch and fixed-bed columns by free and immobilized marine algae Sargassum sp. *Journal of Cleaner Production, 150*, 58–64.

Bayramoğlu, G., Tuzun, I., Celik, G., Yilmaz, M., & Arica, M. Y. (2006). Biosorption of mercury (II), cadmium (II) and lead (II) ions from aqueous system by microalgae Chlamydomonas reinhardtii immobilized in alginate beads. *International Journal of Mineral Processing, 81*(1), 35–43.

Carro, L., Barriada, J. L., Herrero, R., & de Vicente, M. E. S. (2011). Adsorptive behavior of mercury on algal biomass: competition with divalent cations and organic compounds. *Journal of Hazardous Materials, 192*(1), 284–291.

Cataldo, S., Gianguzza, A., Pettignano, A., & Villaescusa, I. (2013). Mercury (II) removal from aqueous solution by sorption onto alginate, pectate and polygalacturonate calcium gel beads. A kinetic and speciation based equilibrium study. *Reactive and Functional Polymers, 73*(1), 207–217.

Cataldo, S., Gianguzza, A., & Pettignano, A. (2016). Sorption of Pd (II) ion by calcium alginate gel beads at different chloride concentrations and pH. A kinetic and equilibrium study. *Arabian Journal of Chemistry, 9*(5), 656–667.

Cazón, J. P., Viera, M., Donati, E., & Guibal, E. (2013). Zinc and cadmium removal by biosorption on Undaria pinnatifida in batch and continuous processes. *Journal of Environmental Management, 129*, 423–434.

Dada, A. O., Olalekan, A. P., Olatunya, A. M., & Dada, O. (2012). Langmuir, Freundlich, Temkin and Dubinin–Radushkevich isotherms studies of equilibrium sorption of Zn^{2+} unto phosphoric acid modified rice husk. *IOSR Journal of Applied Chemistry, 3*(1), 38–45.

Daemi, H., & Barikani, M. (2012). Synthesis and characterization of calcium alginate nanoparticles, sodium homopolymannuronate salt and its calcium nanoparticles. *Scientia Iranica, 19*(6), 2023–2028.

De-Bashan, L. E., & Bashan, Y. (2010). Immobilized microalgae for removing pollutants: review of practical aspects. *Bioresource Technology, 101*(6), 1611–1627.

Dönmez, G. Ç., Aksu, Z., Öztürk, A., & Kutsal, T. (1999). A comparative study on heavy metal biosorption characteristics of some algae. *Process Biochemistry, 34*(9), 885–892.

El-Naggar, A. Y., & Si, C. (2013). Thermal analysis of the modified and unmodified silica gels to estimate their applicability as stationary phase in gas chromatography. *Journal of Emerging Trends in Engineering and Applied Sciences, 4*(1), 144–148.

Esmaeili, A., Saremnia, B., & Kalantari, M. (2015). Removal of mercury (II) from aqueous solutions by biosorption on the biomass of *Sargassum glaucescens* and *Gracilaria corticata*. *Arabian Journal of Chemistry, 8*(4), 506–511.

Figueira, P., Lopes, C. B., Daniel-da-Silva, A. L., Pereira, E., Duarte, A. C., & Trindade, T. (2011). Removal of mercury (II) by dithiocarbamate surface functionalized magnetite particles: application to synthetic and natural spiked waters. *Water Research, 45*(17), 5773–5784.

Gupta, V. K., & Rastogi, A. (2006). Biosorption of lead (II) from aqueous solutions by non-living algal biomass Oedogonium sp. and Nostoc sp.—a comparative study. *Colloids and Surfaces B: Biointerfaces, 64*(2), 170–178.

Gupta, V. K., Rastogi, A., & Nayak, A. (2010). Biosorption of nickel onto treated alga (*Oedogonium hatei*): application of isotherm and kinetic models. *Journal of Colloid and Interface Science, 342*(2), 533–539.

Han, D. S., Orillano, M., Khodary, A., Duan, Y., Batchelor, B., & Abdel-Wahab, A. (2014). Reactive iron sulfide (FeS)-supported ultrafiltration for removal of mercury (Hg (II)) from water. *Water Research, 53*, 310–321.

Jafari, N., & Senobari, Z. (2012). Removal of Pb (II) ions from aqueous solutions by Cladophora rivularis (Linnaeus) hoek. *Scientific World Journal, 2012*.

Ji, L., Xie, S., Feng, J., Li, Y., & Chen, L. (2012). Heavy metal uptake capacities by the common freshwater green alga Cladophora fracta. *Journal of Applied Phycology, 24*(4), 979–983.

Khoramzadeh, E., Nasernejad, B., & Halladj, R. (2013). Mercury biosorption from aqueous solutions by Sugarcane Bagasse. *Journal of the Taiwan Institute of Chemical Engineers, 44*(2), 266–269.

Kumar, J. N., & Oommen, C. (2012). Removal of heavy metals by biosorption using freshwater alga *Spirogyra hyalina*. *Journal of Environmental Biology, 33*(1), 27–31.

Kumar, D., Pandey, L. K., & Gaur, J. P. (2016). Metal sorption by algal biomass: from batch to continuous system. *Algal Research, 18*, 95–109.

Lee, Y. C., & Chang, S. P. (2011). The biosorption of heavy metals from aqueous solution by *Spirogyra* and *Cladophora* filamentous macroalgae. *Bioresource Technology, 102*(9), 5297–5304.

Lohani, M. B., Singh, A., Rupainwar, D. C., & Dhar, D. N. (2008). Studies on efficiency of guava (*Psidium guajava*) bark as bioadsorbent for removal of Hg (II) from aqueous solutions. *Journal of Hazardous Materials, 159*(2), 626–629.

Meitei, M. D., & Prasad, M. N. V. (2013). Lead (II) and cadmium (II) biosorption on Spirodela polyrhiza (L.) Schleiden biomass. *Journal of Environmental Chemical Engineering, 1*(3), 200–207.

Mishra, A., Tripathi, B. D., & Rai, A. K. (2016). Packed-bed column biosorption of chromium(VI) and nickel(II) onto Fenton modified Hydrilla verticillata dried biomass. *Ecotoxicology and Environmental Safety, 132*, 420–428.

Mohan, D., Gupta, V. K., Srivastava, S. K., & Chander, S. (2001). Kinetics of mercury adsorption from wastewater using activated carbon derived from fertilizer waste. *Colloids and Surfaces, A: Physicochemical and Engineering Aspects, 177*(2), 169–181.

Moreno-Garrido, I. (2008). Microalgae immobilization: current techniques and uses. *Bioresource Technology, 99*(10), 3949–3964.

Muzarabani, N., Mupa, M., Gwatidzo, L., & Machingauta, C. (2015). Silica gel matrix immobilized Chlorophyta *Hydrodictyon africanum* for the removal of methylene blue from aqueous solutions: equilibrium and kinetic studies. *African Journal of Biotechnology, 14*(31), 2463–2471.

Oehmen, A., Vergel, D., Fradinho, J., Reis, M. A., Crespo, J. G., & Velizarov, S. (2014). Mercury removal from water streams through the ion exchange membrane bioreactor concept. *Journal of Hazardous Materials, 264*, 5–70.

Parkhurst DL, & Appelo CAJ (2013). Description of input and examples for PHREEQC version 3—a computer program for speciation, batch-reaction, one-dimensional transport, and inverse geochemical calculations. *US Geological Survey Techniques and Methods*, book 6, chap A43, p 497.

Patel, N., Lalwani, D., Gollmer, S., Injeti, E., Sari, Y., & Nesamony, J. (2016). Development and evaluation of a calcium alginate based oral ceftriaxone sodium formulation. *Progress in Biomaterials, 5*(2), 117–133.

Petrovic, A., & Simonic, M. (2016). Removal of heavy metals from drinking water by alginate-immobilized *Chlorella sorokiniana*. *International journal of Environmental Science and Technology, 13*, 1761–1780.

Qiusheng, Z., Xiaoyan, L., Jin, Q., Jing, W., & Xuegang, L. (2015). Porous zirconium alginate beads adsorbent for fluoride adsorption from aqueous solutions. *RSC Advances, 5*(3), 2100–2112.

Rezaee, A., Ramavandi, B., & Ganati, F. (2006). Equilibrium and spectroscopic studies on biosorption of mercury by algae biomass. *Pakistan Journal of Biological Sciences, 9*(4), 777–782.

Rocha, L. S., Lopes, C. B., Henriques, B., Tavares, D. S., Borges, J. A., Duarte, A. C., & Pereira, E. (2014). Competitive effects on mercury removal by an agricultural waste: application to synthetic and natural spiked waters. *Environmental Technology, 35*(6), 661–673.

Ruiz-Marin, A., Mendoza-Espinosa, L. G., & Stephenson, T. (2010). Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. *Bioresource Technology, 101*(1), 58–64.

Shabudeen, S. P. S., Daniel, S., & Indhumathi, P. (2013). Utilizing the pods of *Delonix regia* activated carbon for the removal of mercury (II) by adsorption technique. *Journal of Research in Chemistry and Environment, 3*, 60–65.

Sheikha, D., Ashour, I., & Al-Rub, F. A. (2008). Biosorption of zinc on immobilized green algae: equilibrium and dynamics studies. *The Journal of Engineering Research, 5*(1), 20–29.

Singh, A., Mehta, S. K., Gaur, J. P. (2007). Removal of heavy metals from aqueous solution by common freshwater filamentous algae. *World Journal of Microbiology and Biotechnology, 23*(8), 1115–1120.

Singh, S. K., Dixit, K., & Sundaram, S. (2014). Effect of acidic and basic pretreatment of wild algal biomass on Cr (VI) biosorption. *IOSR Journal of Environmental Science, Toxicology and Food Technology, 8*(5), 38–41.

Song, D., Park, S. J., Kang, H. W., Park, S. B., & Han, J. I. (2013). Recovery of lithium (I), strontium (II), and lanthanum (III) using Ca-alginate beads. *Journal of Chemical & Engineering Data, 58*(9), 2455–2464.

Sud, D., Mahajan, G., & Kaur, M. P. (2008). Agricultural waste material as potential adsorbent for sequestering heavy metal ions from aqueous solutions—a review. *Bioresource Technology, 99*(14), 6017–6027.

Suharso, Buhani, & Sumadi. (2010). Immobilization of *S. duplicatum* supported silica gel matrix and its application on adsorption-desorption of Cu (II), Cd (II) and Pb (II) ions. *Desalination, 263*(1–3), 64–69.

Torres, E., Mata, Y. N., Blazquez, M. L., Munoz, J. A., Gonzalez, F., & Ballester, A. (2005). Gold and silver uptake and nanoprecipitation on calcium alginate beads. *Langmuir, 21* (17), 7951–7958.

Tuzen, M., Sarı, A., Mendil, D., Uluozlu, O. D., Soylak, M., & Dogan, M. (2009). Characterization of biosorption process of As (III) on green algae *Ulothrix cylindricum*. *Journal of Hazardous Materials, 165*(1), 566–572.

Urgun-Demirtas, M., Negri, M. C., Gillenwater, P. S., Nnanna, & Yu, J. (2013). Meeting world's most stringent Hg criterion: a pilot-study for the treatment of oil refinery wastewater using an ultrafiltration membrane process. *Journal of Environmental Management, 117*, 65–75.

Vasudevan, S., Lakshmi, J., & Sozhan, G. (2012). Optimization of electrocoagulation process for the simultaneous removal of mercury, lead, and nickel from contaminated water. *Environmental Science and Pollution Research, 19*, 2734–2744.

Wang, Q., Kim, D., Dionysiou, D. D., Sorial, G. A., & Timberlake, D. (2004). Sources and remediation for mercury contamination in aquatic systems—a literature review. *Environmental Pollution, 131*(2), 323–336.

Wang, S., Vincent, T., Faur, C., & Guibal, E. (2016). Alginate and algal-based beads for the sorption of metal cations: Cu (II) and Pb (II). *International Journal of Molecular Sciences, 17* (9), 1453.

Zeraatkar, A. K., Ahmadzadeh, H., Talebi, A. F., Moheimani, N. R., & McHenry, M. P. (2016). Potential use of algae for heavy metal bioremediation, a critical review. *Journal of Environmental Management, 181*, 817–831.

Zeroual, Y., Moutaouakkil, A., Dzairi, F. Z., Talbi, M., Chung, P. U., Lee, K., & Blaghen, M. (2003). Biosorption of mercury from aqueous solution by *Ulva lactuca* biomass. *Bioresource Technology, 90*(3), 349–351.