Live and lyophilized fungi-algae pellets as novel biosorbents for gold recovery: Critical parameters, isotherm, kinetics and regeneration studies

Na Shen* and Evans M.N. Chirwa

Department of Chemical Engineering, University of Pretoria, Pretoria 0028, South Africa

Abstract

This study aimed to evaluate the potential of live and lyophilized fungi-algae pellets as biosorbents for gold recovery and their regeneration ability. The optimum conditions determined by Taguchi method were 1 g/L co-pellets, 9-10 mm size at 250 rpm of agitation speed and pH 3.5 and 2.0 for live and lyophilized co-pellets, respectively. The porous characteristics of fungi-algae pellets played an important role on gold adsorption. Lyophilized co-pellets achieved adsorption capacity of 112.36 mg/g which were comparable with some synthesized granular adsorbents and performed better than the live co-pellets due to more cell-wall polysaccharides involved in gold interaction. 97.77 % of gold was selectively absorbed by the lyophilized co-pellets from multi-metal wastewater in column reactor. This study may provide new insights into the application of fungi-algae pelletized reactor in bioremediation of contaminated wastewater by precious metals and their recovery and the in-situ regeneration of biosorbents.

Keywords: Gold; Fungi-algae pellets; Lyophilized pellets; Taguchi method; Pelletized reactor

1. Introduction

The discard and discharge of gold from secondary sources such as electronic and electrical scraps and industrial wastewater has created a severe contamination of natural water resources and soil. Biosorption is an efficient and economical technology for remediation of

* Corresponding author.
E-mail address: nashen2016@gmail.com

contaminated water by heavy metals and recovery of precious metals from those secondary sources by biomass such as bacteria, fungi, algae, agricultural and industrial by-products (Syed, 2012). Especially, microalgae due to their easy availability and unique surface properties have been proved as promising biosorbents (Bindschedler et al., 2017; Das, 2010). It has been well studied that ion exchange is the most important mechanism in biosorption of heavy/precious metal ions by algal biomass. The availability of binding groups on the algal cell surface i.e. hydroxyl, carboxyl, sulphuryl, amine, phosphate, carbohydrate, etc., also involved in the biosorption capacity (Keyvan et al., 2016).

However, the costly and energy intensive separation of those tiny algal cells from diluted wastewater restrict their large-scale application. Bioflocculation as an environmental-friendly and cost-efficient strategy for microalgae harvest has attracted increasing attention (Alam et al., 2016). Immobilization of algal cells on fungal hyphae by co-culture of microalgae cells with filamentous fungi has been proposed as one of efficient bio-flocculating methods (Alam et al., 2016; Chen et al., 2018; Muradov et al., 2015; Zhou et al., 2012). Although the studies on treatment of municipal wastewater (Zhou et al., 2012) and arsenic bioremediation (Li et al., 2019) by the fungi-algae pellets have been reported, their applications in wastewater treatment are still quite few. Especially, the application of fungi-algae pellets as biosorbents in recovery of precious metals with the advantage of easy separation from wastewater by their self-flocculating sedimentation in column reactor has never been reported yet.

In addition, some synthesized granular adsorbents such as resins and microcapsules, possess desirable adsorption capacity in recovery of precious metals by chemical modification or crosslinking process (Fujiwara et al., 2007; Kiyoyama et al., 2008). These synthesized adsorbents normally have a size of several hundred micrometres that indeed benefit their separation from wastewater, but their cost of production and processing is still the most discussed draw back (Adhikari et al., 2008; Fujiwara et al., 2007). Substantial studies found

that fungi-algae pellets could be formed by co-culturing of microalgae and filamentous fungal spores (Li et al., 2019; Muradov et al., 2015; Zhou et al., 2012). Recently, another promising alternative has been proposed by inducing pre-formed fungal pellets into microalgal culture which took less in process time and glucose input (Chen et al., 2018). Especially, the short process time is very important to the efficient desorption before the gold ions reduced to the metallic gold.

Hence, in this study, the live and lyophilized co-pellets formed by immobilizing microalgae on pre-formed fungal pellets were used as biosorbents for gold recovery from wastewater. The optimum conditions for gold adsorption were determined by Taguchi method. The adsorption behaviour of co-pellets and the change of surface characteristics after adsorption were studied and their regeneration ability using thiourea and cyanide waste was evaluated. The application of fungi-algae pellets in treatment of gold-bearing wastewater was conducted in the column-type SBR. The results may provide new insights into the recovery of precious metals from wastewater by the cost-efficient and eco-friendly fungi-algae pellets with good sedimentation property.

2. Materials and Methods

2.1. Microalgae and fungi

Microalga *Tetradesmus obliquus AS-6-1* was initially isolated from freshwater located in Southern Taiwan (Zhang et al., 2016). The pure strain was cultured in sterile Blue-Green (BG-11) medium (Kim et al., 2011) at 25±2 °C with a light intensity of approximately 60 μmol m^{-2} s^{-1}. Filamentous fungi *Aspergillus niger* CMW 40914 (International Collection Number CBS133818) was obtained from the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa. The strain was grown in incubator at 28 °C for 7 days on slant with potato dextrose agar (PDA) for reactivation, and then collected

and diluted in 50 mL sterile water prior to use. The spore suspension was used as the inoculums for the fungal pellet formation and the number of spores was counted by optical microscope (Chen et al., 2018).

2.2. Formation of fungi-algae pellets

A. niger spore suspension (7.2×10^3 spores/mL) was inoculated into potato dextrose broth (PDB) at pH 4 and grown in an incubator with an orbital shaker of 120 rpm, at 28 °C for 4-5 days. Then the pre-formed fungal pellets at determined sizes were added into *T. obliquus AS-6-1* culture at pH 5.0 with the fungi: algae ratio of 1:2 in an orbital shaker of 200 rpm at 25 °C. After 3 h' shaking, almost all the algal cells were immobilized on the fungal pellets. The formed fungi-algae pellets (co-pellets) rinsed thoroughly with deionized water were lyophilized using the freeze dryer (Martin Christ Alpha 3-4 LSCbasic) and preserved in dry conditions as adsorbents prior to use.

2.3. Determination of optimum conditions by Taguchi method

Taguchi method is a systematic and efficient approach to optimise the process parameters in view of performance and cost (Soleimani and Kaghazchi, 2008). In the present study, the optimization of parameters for gold adsorption using live and lyophilized co-pellets was determined by Taguchi method. Four sets of three-level major adsorption parameters were selected to form the eight degrees of freedom in Table 1. According to Taguchi method, an orthogonal array L9 with four columns and nine rows were used for the optimization experiments shown in Table 2. All the experiments were conducted in 100 mL of $HAuCl_4$ solution with an initial concentration of 30 mg/L and then shaken for 6 h at 25±2 °C.

2.4. Kinetic and isotherm studies

Kinetic adsorption by fungi-algae pellets at the optimum conditions was analysed using Lagergren's pseudo first-order, pseudo second-order and intraparticle diffusion models

(Zheng et al., 2009) which were then simulated using the Computer Program for the Identification and Simulation of Aquatic Systems AQUASIM 2.01 (AQUASIM™, EAWAG, Dübendorf, Switzerland). Langmuir, Freundlich and Temkin isotherm models (Zheng et al., 2009) were applied to analyse the equilibrium adsorption conducted in 100 mL of $HAuCl_4$ solution with an initial concentration from 80-200 mg/L after 6 h under the optimum conditions at 25±2 °C.

2.5. Desorption and regeneration

Thiourea and NaCN collected from the gold mining wastewater discharged into tailing (Sibanye Stillwater, South Africa) were used as desorbing agents to strip the adsorbed gold ions from loaded lyophilized co-pellets. Biosorption experiments were conducted in triplicates at optimum conditions for an initial Au(III) concentration of 30 mg/L within 1 h at 25±2 °C. The subsequent desorption was carried out in 50 mL of 1 M thiourea and NaCN waste (17 mg/L free CN- in mining wastewater) at determined pH (adjusted by HNO_3/NaOH) under 25±2 °C and shaken for 4 h. The regenerated co-pellets would be used as adsorbents for the next cycle. Each new cycle of adsorption was carried out by supplementing 30 mg/L $HAuCl_4$.

2.6. Fungi-algae pelletized reactor

The column-type SBR (2.5 of height/diameter ratio with height 20 cm and inner diameter 8 cm) was packed with l.0 g of 9-10 mm lyophilized co-pellets in a working volume of 1 L wastewater from different sources (pH=2.0, 25±2 °C). The lab wastewater was collected from the experimental waste of gold adsorption/desorption in our lab and the gold mining wastewater was modified by adding standard stock solution of chloroauric acid (the original gold concentration in mining wastewater before modification was negligible with only 2 mg/L). PCB wastewater was simulated from the printed circuit boards (PCB) manufacturing

factory (Han et al., 2017). After 1 h adsorption, samples were taken from the outlets at 250 mL, 500 mL and 750 mL, respectively, and the residual wastewater was discharged from the outlet at the bottom. Subsequently, the co-pellets were washed thoroughly by the deionized water and regenerated in-situ by 500 mL of 1 M thiourea at pH 0.7 in SBR.

2.7. Analytical methods

Scanning Electron Microscopy/Energy Dispersive Spectroscopy (SEM_EDS) (JOEL JSM 5800LV, Tokyo, Japan) was used to examine cell surface morphology and elemental composition before and after adsorption for the tested pellets. Fourier Transform Infrared Spectroscopy (FTIR-Nicolet iS5, Thermo, South Africa) was used to describe the formation of co-pellets (Shen and Chirwa, 2019). The amount of gold was quantified using Atomic Absorption Spectrometer (AAS Perkin Elmer AAnalyst 400). The amount of multiple metals in wastewater before and after adsorption was quantified by Inductively Coupled Plasma (ICP, Spectro Arcos FHS12, Boschstroisse, Germany). The free CN^- in the gold-mining wastewater was determined using the standard titrimetric method (Obiri et al., 2007) and the thiourea measurement (Blagrove and Gruen, 1971) in the lab wastewater was used by a UV/V spectrophotometer (WPA, light wave II, Labotech, South Africa) at a wavelength of 236 nm. All experiments were triplicate and the error bars represented the mean's standard deviations of the triplicates.

3. Results and Discussion

3.1. Optimal conditions of gold adsorption

The studies on the effect of surface porosity on adsorption have been well studied on the synthesized or modified porous carbons, but on the completely biological adsorbents are rarely reported. The average percentage of final adsorption efficiency was selected as a response of the corresponding parameter with Taguchi method. The adsorption efficiency

increased with an increasing diameter of the live and lyophilized co-pellets as present in Fig. 1a. Whereas, (Soleimani and Kaghazchi, 2008) reported that gold adsorption efficiency decreased with an increase in size of active carbon from 0.3-1.7 mm. This may be due to the significance of porous property to fungi-algae pellets at size of 3-10mm but surface area was more important to the fine active carbon.

The SEM results showed that the live and lyophilized co-pellets had fibrous and porous surface structures. The co-pellet at small size of 3-4 mm was compact and tight, while the pellet surface at bigger size of 9-10 mm were comparably loose and even the inner side can be observed through the big porous surface. The loose and big porous surface property of bigger co-pellets can be considered as a factor providing an increase in their total surface area and a decrease in the diffusional resistance (Bayramoglu et al., 2009). This may explain why the bigger co-pellets performed better in adsorption as shown in Fig. 1a. The porous characteristics of fungi-algae pellets played an important role in the gold adsorption.

In addition, the formation of fungal pellets prefers the acidic environments while microalgae cells are usually stable and freely-suspended in alkaline suspension. Hence, it is important to find out the optimal pH for the compatibility of fungi and microalgae existing in the co-pellets for the effective gold adsorption. Based on the results in Fig. 1 by Taguchi method with the higher-the-better performance characteristic, the optimum conditions of gold adsorption by live and lyophilized co-pellets were 9-10 mm of pellet size, 1 g/L of adsorbent dosage, 250 rpm of agitation speed, and pH 3.5 for the live fungi-algae pellets and pH 2.0 for the lyophilized ones.

3.2. Effect of contact time and kinetic study

The profile of Au(III) adsorption by live and lyophilized co-pellets from 0-6 h at the optimum conditions was depicted in Fig. 2a. The adsorption capacity gradually increased with time and reached the equilibrium within approximately 5 h. Hence 6 h was enough to obtain the

equilibrium for the further experiments. Kinetic data was described with pseudo-first/second-order and intraparticle diffusion models at the optimum conditions (Fig. 2b, 2c). It was found that pseudo-second-order model better represented the adsorption kinetics for live (R^2 = 0.9870) and lyophilized co-pellets (R^2 = 0.9966). Additional evidence for the better fit to pseudo-second-order model was provided with no pronounced difference between experimental $q_{e,exp}$ and calculated $q_{e,cal}$ data (Table 3).

For the pelletized adsorbents, the mass transfer limitation is important for the surface adsorbed Au(III) transferred into the interior of adsorbent. Here the intraparticle diffusion model was used to describe the Au(III) adsorption by live co-pellets which could be divided into two stages (Fig. 2d and Table 4). The first stage I with the higher rate of reaction (6.46 mg/g $min^{1/2}$) was surface adsorption which occurred fast in the first 5-30 minutes due to the abundance in the available active functional groups. In the second stage II (0.5-6 h), the adsorption rate decreased to 2.48 mg/g $min^{1/2}$ which may be due to the occupied binding sites and the intraparticle diffusion limitation.

However, Au(III) adsorption by lyophilized co-pellets involved three stages (Fig. 2d and Table 4). In stage I (5-30 min), the abundant availability of vacant binding sites on lyophilized co-pellets may account for the highest adsorption rate of 7.85 mg/g $min^{1/2}$. In stage II (0.5-3 h), the adsorption rate decreased to 4.64 mg/g $min^{1/2}$ may be due to the decrease of available binding sites and the intraparticle diffusion limitation. The further significant decrease in adsorption rate to 0.61 mg/g $min^{1/2}$ with equilibrium in adsorption capacity in the last stage III (3-6 h) could be due to the saturation of binding sites on lyophilized co-pellets. Although the multi-linear intraparticle diffusion model with different rate constants implied that more than one steps controlled the adsorption process (Fig. 2d), the preference of pseudo-second-order model indicated that the rate-controlling step is a

chemical adsorption process between Au(III) and functional groups on live and lyophilized co-pellets (Lin et al., 2019).

3.3. Effect of initial Au(III) concentration and isotherm study

The adsorption capacity improved with an increase in Au(III) concentrations from 80 to 200 mg/L as presented in Fig. 3 and obtained the maximum adsorption capacity of 98.60 mg/g and 110.87 mg/g for live and lyophilized co-pellets, respectively. This may be due to the abundant availability of vacant binding sites at lower concentration and then the active binding groups became completely saturated with increased Au(III) concentration (Birungi and Chirwa, 2015).

The values of R^2 in Table 5 showed that the fit of isotherm models to the Au(III) uptake by live and lyophilized co-pellets followed the sequence: Langmuir > Freundlich > Temkin. In addition, the experimental maximum adsorption capacity was much closed to the maximum adsorption capacity q_m from Langmuir model of 104.17 and 112.36 mg/g for live and lyophilized co-pellets, respectively. Hence, the adsorption isotherms of Au(III) were described well by the Langmuir model, indicating the monolayer adsorption of Au(III) by the possible uniform distribution of binding sites on the cell surface of live and lyophilized co-pellets. Furthermore, the lyophilized co-pellets are considered as preferable biosorbents for Au(III) adsorption than the live co-pellets because of the higher q_m and the ease of storage for their large-scale application.

The essential characteristics of Langmuir were expressed using a dimensionless constant separation factor(Birungi and Chirwa, 2015), R_L.

$$R_L = \frac{1}{1+(1+b_L C_0)} \quad (1)$$

where b_L constant related to the Langmuir constant b; C_o is the initial concentration (mg/L). R_L values indicate the adsorption to be unfavourable when $R_L>1$, favourable when $0< R_L<1$, and irreversible when $R_L =0$. All the calculated R_L were shown in Fig. 4 for live and lyophilized co-pellets ($0<R_L<0.08$) and found to be favourable for Au(III) adsorption, which further implied the high potential of fungi-algae pellets as biosorbents for gold adsorption.

Compared to some synthesized granular adsorbents by crosslinking and chemical modification possessing high adsorption capacity (Fujiwara et al., 2007; Gamez et al., 2003; Kiyoyama et al., 2008; Nguyen et al., 2010; Soleimani and Kaghazchi, 2008), the biological granular adsorbents of live and lyophilized co-pellets in this study showed their comparable adsorption capacity and exhibited higher capacity than other biomass (Al-Saidi, 2016; Mata et al., 2009; Nakajima, 2003) in Au(III) adsorption. Furthermore, due to their big size of 9-10 mm, the ease of separation of co-pellets from diluted wastewater without the energy-intensive separation methods such as centrifugation and flotation further demonstrated that the live and lyophilized fungi-algae pellets are efficient and promising biosorbents for Au(III) adsorption.

The adsorption capacity fitting to pseudo-second-order model at 100 mg/L of initial Au (III) concentration under equal operating conditions followed the sequence: lyophilized (*T. obliquus AS-6-1+A. niger*) pellets (94.34 mg/g) > live *A. niger* pellets (85.47 mg/g) > lyophilized *A. niger* pellets (84.03 mg/g) > lyophilized *T. obliquus AS-6-1* (81.97 mg/g) > live *T. obliquus AS-6-1* (80 mg/g) = live (*T. obliquus AS-6-1+A. niger*) pellets (80 mg/g). The lyophilized fungi-algae pellets exhibited higher adsorption capacity than the individual microalgae *T. obliquus AS-6-1* and fungi *A. niger* alone, further indicating the superiority of lyophilized co-pellets as adsorbent for gold recovery.

3.4. Surface change of fungi-algae pellets after adsorption

Approximately 3 h after adsorption initiation, the colour of live co-pellets started to turn dark reddish. Whereas, the colour of lyophilized co-pellets had no pronounced change with only few pellets changed to reddish even after 6 h. (Vijayaraghavan et al., 2011) and (Castro et al., 2013) also observed the similar colour change using brown alga and red alga and proposed that it was due to the possible bioreduction of gold and the presence of gold nanoparticles. In addition, the colour change occurred slowly on lyophilized co-pellets implied that the reduction of surface adsorbed Au(III) to Au(0) was slower than that occurred on live co-pellets (Gao et al., 2017).

FTIR analysis of co-pellets showed the presence of hydroxyl (3270 cm^{-1}), asymmetric CH_2CH_3 of lipids (2923 cm^{-1}), carboxylic (1639 cm^{-1}), N-H bends due to amide II (1545 cm^{-1}) groups of proteins and C-O stretching (1027 cm^{-1}) from polysaccharides might be the major functional groups on their cell surfaces (Li et al., 2019; Salman et al., 2010). (Das, 2010) concluded that hydroxyl groups present in the algal polysaccharides were involved in gold biosorption and bioreduction using algae alone. The protonated carbonyl and carboxyl groups and the involvement of C–O and C–N bonds played a key role in gold biosorption by fungi alone. The significant increase in absorbance of hydroxyl stretching at 3270 cm^{-1} after adsorption might be due to the water adsorbed onto the lyophilized co-pellets. The decrease in absorbance of C-O stretching (1027 cm^{-1}) from polysaccharides on lyophilized co-pellets after adsorption and the EDS results (0 Wt% of Au before adsorption and 38.7 Wt% Au after adsorption) confirmed the cell-wall polysaccharides involved in the adsorption process of Au(III).

However, the absorbance of C-O stretching at 1027 cm^{-1} on live co-pellets increased after adsorption, indicating the increase of corresponding cell-wall polysaccharides. Exopolysaccharides (EPSs) are metabolic products that accumulate on the microbial cell

surface and provide protection to the cells by stabilizing membrane structure against the harsh external environment (Li et al., 2019). EPSs, may excreted from both microalgae and fungi, are mainly composed of polysaccharides, proteins, nucleic acids and lipids (Li et al., 2019; Xiao and Zheng, 2016). Hence, increase in polysaccharide peaks may be due to the EPSs released from the live fungi-algae pellets under the unfavourable conditions caused by the high-level gold ions added (Schröfel et al., 2011). The significant increase in absorbance at 1027 cm^{-1} that corresponds to the C-O bonding of polysaccharides which take part in gold interaction may explain why the lyophilized co-pellets performed better in Au(III) adsorption.

3.5. Thiourea and NaCN waste as desorbing agents

Thiourea and CN- have been used as efficient desorbing agents to strip the adsorbed gold ions from adsorbents. Although it is not advisable to use CN- reagent due to its acute toxicity (Fujiwara et al., 2007), the use of cyanide waste as eluent could be an economical way to desorb the loaded gold ions from adsorbents. In this study, the potential of thiourea and gold mining wastewater after discharged into tailing containing 17 mg/L CN- were evaluated as eluents for stripping gold from loaded lyophilized co-pellets. Desorption performance of thiourea was decreased with an increase in pH from 0.5 to 2.0, while an increase in desorption efficiency was observed with increasing pH to 8.0 for CN- as desorbing agent (Fig. 5). The best performance of approximately 100% desorption efficiency achieved by 1 M thiourea at pH 0.5-0.7 and 76.4 % desorption efficiency obtained by 17 mg/L CN- at pH 8.0. Hence, 1 M thiourea at pH 0.7 was selected as desorbing agent for gold recovery in the further studies.

Interestingly, desorption efficiency was decreased with time which may be due to the adsorption of gold–thiourea and gold–cyanide complex, e.g., $Au[CS(NH_2)_2]^+$ and $Au(CN)_2^-$ or $Au(CN)_4^-$ by the cell-wall binding groups (Greene et al., 1986; Savvaidis, 1998). Further desorption experiments were carried out for 0.5 h, the short time essential for industrial

application. However, the gold-mining wastewater containing considerable amount of CN^- with comparable good desorption capacity showed that it is still a promising eluent for the recovery of gold or other precious metals. Further studies are required to optimise the desorption process using CN^- from gold-mining wastewater before discharged into tailing.

3.6. Adsorption potential of regenerated fungi-algae pellets

The potential of regenerated co-pellets in Au(III) adsorption was evaluated using different acidic thiourea (Fig. 6). The live and lyophilized co-pellets showed their efficient adsorption capacity before thioure treatment. However, the adsorption efficiency of regenerated co-pellets by thiourea at pH 0.7 dropped below 20 % may be due to the disintegration of co-pellets in strong acidic thiourea. Although the co-pellets stayed intact in an increasing pH of thiourea to 2.0, the regenerated co-pellets still performed undesirably in adsorption. In the case of l-lysine modified crosslinked chitosan resin as adsorbent (Fujiwara et al., 2007), the synthesize adsorbent can be reused after desorption with 0.7 M thiourea-2 M HCl without loss of the adsorption capacity due to the enhancement of the stability against acidic or alkaline solutions by the crosslinking step. As reported by (Acheampong and Ansa, 2017), the adsorption capacity in some cases may be improved after desorption treatment, while in other cases there may be a loss of sorption capacity and even a complete disintegration of the biosorbent. It must be admitted that compared with those synthesized adsorbents, the biological adsorbents of fungi-algae pellets in this study are intolerant against strong acidic solutions.

3.7. Wastewater treatment in fungi-algae pelletized reactor

The potential of fungi-algae pellets as adsorbents for gold recovery was evaluated in treatment of gold-containing wastewater from different sources. As discussed above, the lyophilized co-pellets with higher adsorption capacity were used to adsorb Au(III) from lab,

gold-mining and PCB wastewater in 1 L of column-type SBR. After 1 h adsorption, the pellets could easily settle at the bottom (for live co-pellets) or suspend on the top (for lyophilized co-pellets) of reactor in few seconds without those energy-intensive separating method such as centrifuge and flotation. The lyophilized fungi-algae pellets performed in PCB wastewater indicated that they have high selectivity and specificity to Au(III) with good efficiency of 97.77 % in adsorption. In contrast, the adsorption efficiency of other metals in the PCB wastewater such as Ni and Co was quite low (Table 6).

In terms of Au(III) adsorption from lab and mining wastewater, the co-pellets did not perform as well as that in the PCB wastewater (Table 6). This may be due to the presence of thiourea and CN^- used as desorbing agents which could form gold–thiourea and gold–cyanide complex and interfere the Au(III) adsorbed onto the cell surface of co-pellets. On the other hand, although the K^+ concentration (354.4 mg/L) was almost 12 times higher than Au(III) (30 mg/L) in PCB wastewater, the gold adsorption was not affected by the presence of high level of K^+. This is consistent with reports by (Han et al., 2017) and (Gamez et al., 2003) who proposed that K^+, Mg^{2+} and Ca^{2+} rarely affected the gold adsorption. Almost 98 % adsorbed gold ions on fungi-algae pellets could be stripped off by 500 mL of 1 M thiourea at pH 0.7 within 0.5 h in SBR (data not shown), implying that the good potential of lyophilized co-pellets as biosorbents for in-situ gold recovery from wastewater in the future industrial application.

3.8. Economic aspects of fungi-algae pellets as biosorbent

The fungi-algae pellets are easily formed by adding the fungal pellets into algal culture under the proper conditions. These co-pellets as biosorbents for gold recovery have comparably high adsorption capacity and excellent precipitation property in wastewater. Especially the lyophilized co-pellets with the ease of storage and higher performance in gold adsorption are

proposed to be efficient and promising adsorbents in the industrial application. Although their reusability is not as satisfactory as those synthesised granular adsorbents, e.g., the l-lysine modified cross-linked chitosan resin (Fujiwara et al., 2007), their product cost is inexpensive and their process in gold recovery reduces the secondary pollution which can meet the targets of circular economy and bioresource reutilization. Nevertheless, the limitation of reusability of fungi-algae pellets as biosorbents should be improved by developing moderate desorbing agents or improving the stability of co-pellets against acidic or alkaline solutions. Also, advanced methods allowing low-cost and high-efficiency production of the fungi-algae pellets and simultaneous gold adsorption should be developed to realize the industrial application of this technology.

4. Conclusions

Due to the comparable adsorption capacity with synthesized granular adsorbents, the fungi-algae pellets formed by immobilizing microalgae on fungal pellets showed their great potential for gold recovery, especially the lyophilized co-pellets with ease of storage and higher adsorption capacity than the live co-pellets. More studies are still required to improve the reusability of fungi-algae pellets as biosorbents for the in-situ gold recovery in their industrial application.

Acknowledgements

The authors would like to thank the Sedibeng Water, South Africa and the Water Utilization and Environmental Engineering Division at the University of Pretoria for financial and logistical support, and appreciate the kind help of FABI, University of Pretoria, for providing the fungi species, and Professor Xinqing Zhao in Shanghai Jiao Tong University and Professor Jo-Shu Chang in National Cheng Kung University for providing the microalgae strains.

Appendix A. Supplementary data

E-supplementary data for this work can be found in e-version of this paper online.

Reference

1. Adhikari, C.R., Parajuli, D., Inoue, K., Ohto, K., Kawakita, H., Harada, H., 2008. Recovery of precious metals by using chemically modified waste paper. Environ. Sci. Technol. 42, 5486–5491.

2. Al-Saidi, H.M., 2016. The fast recovery of gold (III) ions from aqueous solutions using raw date pits : Kinetic , thermodynamic and equilibrium studies. J. Saudi Chem. Soc. 20, 615–624.

3. Alam, A., Vandamme, D., Chun, W., Zhao, X., Foubert, I., Wang, Z., Muylaert, K., Yuan, Z., 2016. Bioflocculation as an innovative harvesting strategy for microalgae. Rev. Environ. Sci. Biotechnol. 15, 573–583.

4. Bayramoglu, G., Gursel, I., Tunali, Y., Arica, M.Y., 2009. Biosorption of phenol and 2-chlorophenol by Funalia trogii pellets. Bioresour. Technol. 100, 2685–2691.

5. Bindschedler, S., Vu Bouquet, T.Q.T., Job, D., Joseph, E., Junier, P., 2017. Fungal Biorecovery of Gold From E-waste, Adv. App. Microbiol. 99, 53–81.

6. Birungi, Z.S., Chirwa, E.M.N., 2015. The adsorption potential and recovery of thallium using green micro-algae from eutrophic water sources. J. Hazard. Mater. 299, 67–77.

7. Blagrove, R.J., Gruen, L.C., 1971. Spectrophotometric determination of thiourea. Mikrochim. Acta. 59, 639–643.

8. Castro, L., Castro, L., Blázquez, M.L., Muñoz, J.A., González, F., Ballester, A., 2013. Biological synthesis of metallic nanoparticles using algae. IET Nanobiotechnology 7, 109–116.

9. Chen, J., Leng, L., Ye, C., Lu, Q., Addy, M., Wang, J., Liu, J., Chen, P., Ruan, R., Zhou, W., 2018. A comparative study between fungal pellet- and spore-assisted microalgae harvesting methods for algae bioflocculation. Bioresour. Technol. 259, 181–190.

10. Das, N., 2010. Hydrometallurgy Recovery of precious metals through biosorption — A review. Hydrometallurgy 103, 180–189.

11. Fujiwara, K., Ramesh, A., Maki, T., Hasegawa, H., Ueda, K., 2007. Adsorption of platinum (IV), palladium (II) and gold (III) from aqueous solutions onto l-lysine modified crosslinked chitosan resin. J. Hazard. Mater. 146, 39–50.

12. Gamez, G., Gardea-Torresdey, J.L., Tiemann, K.J., Parsons, J., Dokken, K., Yacaman, M.J., 2003. Recovery of gold(III) from multi-elemental solutions by alfalfa biomass. Adv. Environ. Res. 7, 563–571.

13. Gao, X., Zhang, Y., Zhao, Y., 2017. Biosorption and reduction of Au (III) to gold nanoparticles by thiourea modified alginate. Carbohydr. Polym. 159, 108–115.

14. Greene, B., Hosea, M., McPherson, R., Henzl, M., Alexander, M.D., Darnall, D.W., 1986. Interaction of Gold(I) and Gold(III) Complexes with Algal Biomass. Environ. Sci. Technol. 20, 627–632.

15. Han, Y.L., Wu, J.H., Cheng, C.L., Nagarajan, D., Lee, C.R., Li, Y.H., Lo, Y.C., Chang, J.S., 2017. Recovery of gold from industrial wastewater by extracellular proteins obtained from a thermophilic bacterium Tepidimonas fonticaldi AT-A2. Bioresour. Technol. 239, 160–170.

16. Keyvan, A., Ahmadzadeh, H., Farhad, A., Moheimani, N.R., Mchenry, M.P., 2016. Potential use of algae for heavy metal bioremediation , a critical review. J. Environ. Manage. 181, 817–831.

17. Kim, D.G., La, H.J., Ahn, C.Y., Park, Y.H., Oh, H.M., 2011. Harvest of Scenedesmus sp. with bioflocculant and reuse of culture medium for subsequent high-density cultures. Bioresour. Technol. 102, 3163–3168.

18. Kiyoyama, S., Maruyama, T., Kamiya, N., Goto, M., 2008. Immobilization of proteins into microcapsules and their adsorption properties with respect to precious-metal ions. Ind. Eng. Chem. Res. 47, 1527–1532.

19. Li, B., Zhang, T., Yang, Z., 2019. Immobilizing unicellular microalga on pellet-forming filamentous fungus: Can this provide new insights into the remediation of arsenic from contaminated water? Bioresour. Technol. 284, 231–239.

20. Lin, G., Hu, T., Wang, S., Xie, T., Zhang, L., Cheng, S., Fu, L., Xiong, C., 2019. Selective removal behavior and mechanism of trace Hg(II) using modified corn husk leaves. Chemosphere 225, 65–72.

21. Mata, Y.N., Torres, E., Blázquez, M.L., Ballester, A., González, F., Munoz, J.A., 2009. Gold(III) biosorption and bioreduction with the brown alga Fucus vesiculosus. J. Hazard. Mater. 166, 612–618.

22. Acheampong M.A., Ansa E.D.O., 2017. Low-Cost Technologies for Mining Wastewater Treatment. J. Environ. Sci. Eng. B 6, 391–405.

23. Muradov, N., Taha, M., Miranda, A.F., Wrede, D., Kadali, K., Gujar, A., Stevenson, T., Ball, A.S., Mouradov, A., 2015. Fungal-assisted algal flocculation: Application in wastewater treatment and biofuel production. Biotechnol. Biofuels 8.

24. Nakajima, A., 2003. Accumulation of gold by microorganisms. World J. Microbiol. Biotechnol. 19, 369–374.

25. Nguyen, N. V., Jeong, J., Jha, M.K., Lee, J.C., Osseo-Asare, K., 2010. Comparative

studies on the adsorption of Au(III) from waste rinse water of semiconductor industry using various resins. Hydrometallurgy 105, 161–167.

26. Obiri, S., Dodoo, D.K., Okai-Sam, F., Essumang, D.K., 2007. Determination of free cyanide and total cyanide concentration in surface and underground waters in Bogoso and its surrounding aereas in Ghana. Chem. Soc. Ethiop. 21, 213–220.

27. Salman, A., Tsror, L., Pomerantz, A., Moreh, R., Mordechai, S., Huleihel, M., 2010. FTIR spectroscopy for detection and identification of fungal phytopathogenes. Spectroscopy 24, 261–267.

28. Savvaidis, I., 1998. Recovery of gold from thiourea solutions using microorganisms. BioMetals 11, 145–151.

29. Schröfel, A., Kratošová, G., Bohunická, M., Dobročka, E., Vávra, I., 2011. Biosynthesis of gold nanoparticles using diatoms-silica-gold and EPS-gold bionanocomposite formation. J. Nanoparticle Res. 13, 3207–3216.

30. Shen, N., Chirwa, E.M.N., 2019. Short-term adsorption of gold using self-flocculating microalga from wastewater and its regeneration potential by bio-flocculation. J. Appl. Phycol. 31, 1783–1792.

31. Soleimani, M., Kaghazchi, T., 2008. Adsorption of gold ions from industrial wastewater using activated carbon derived from hard shell of apricot stones – An agricultural waste. Bioresour. Technol. 99, 5374–5383.

32. Syed, S., 2012. Recovery of gold from secondary sources-A review. Hydrometallurgy 115–116, 30–51.

33. Vijayaraghavan, K., Mahadevan, A., Sathishkumar, M., Pavagadhi, S., Balasubramanian, R., 2011. Biosynthesis of Au(0) from Au(III) via biosorption and bioreduction using

brown marine alga Turbinaria conoides. Chem. Eng. J. 167, 223–227.

34. Xiao, R., Zheng, Y., 2016. Overview of microalgal extracellular polymeric substances (EPS) and their applications. Biotechnol. Adv. 34, 1225–1244.

35. Zhang, X., Zhao, X., Wan, C., Chen, B., Bai, F., 2016. Efficient biosorption of cadmium by the self-flocculating microalga Scenedesmus obliquus AS-6-1. Algal Res. 16, 427–433.

36. Zheng, H., Liu, D., Zheng, Y., Liang, S., Liu, Z., 2009. Sorption isotherm and kinetic modeling of aniline on Cr-bentonite. J. Hazard. Mater. 167, 141–147.

37. Zhou, W., Cheng, Y., Li, Y., Wan, Y., Liu, Y., Lin, X., Ruan, R., 2012. Novel Fungal Pelletization-Assisted Technology for Algae Harvesting and Wastewater Treatment. Appl. Biochem. Biotechnol. 167, 214–228.

FIGURE CAPTIONS

Figure 1. Average responses at different levels of (a) co-pellet size, (b) dosage of co-pellets, (c) pH of gold solution and (d) agitation speed.

Figure 2. (a) Effect of contact time on the adsorption capacity of co-pellets at optimum conditions. Experimental data of (b) live co-pellets and (c) lyophilized co-pellets fitted to the kinetic models. (d) Kinetic stages of intraparticle diffusion models for co-pellets.

Figure 3. Effect of initial Au(III) concentrations on the adsorption capacity of co-pellets at optimum conditions.

Figure 4. Profile of R_L of live and lyophilized co-pellets with initial gold concentrations.

Figure 5. Percentage of adsorbed gold desorbed from lyophilized co-pellets using (a) thiourea and (b) mining wastewater containing CN^- at different pH.

Figure 6. Au(III) adsorption efficiency of regenerated (a) live and (b) lyophilized co-pellets by thiourea at different pH.

Table 1 Gold adsorption parameters and their levels with Taguchi method.

Parameters	Unit	L1	L2	L3
Adsorbent size	mm	6-7	9-10	3-4
Biomass	g/L	0.1	0.3	1.0
Agitation speed	rpm	100	150	250
pH		2.0	3.5	5.0

Table 2 Arrangement of adsorption factors in L9 orthogonal array.

Experiments	Adsorbent size (mm)	Biomass (g/L)	Agitation speed (rpm)	pH
t1	6-7	0.1	100	2.0
t2	6-7	0.3	150	3.5
t3	6-7	1.0	250	5.0
t4	9-10	0.1	150	5.0
t5	9-10	0.3	250	2.0
t6	9-10	1.0	100	3.5
t7	3-4	0.1	250	3.5
t8	3-4	0.3	100	5.0
t9	3-4	1.0	150	2.0

Table 3 Comparison of kinetic parameters obtained from pseudo first and second order and intraparticle diffusion models[a] for co-pellets at optimum adsorption conditions.

Parameters	Live co-pellets	Lyophilized co-pellets
Pseudo first order		
k_1	0.0102	0.0133
q_e (mg/g)	66.51	67.02
R^2	0.9242	0.9569
Pseudo second order		
k_2	0.0005	0.0006
$q_{e,cal}$ (mg/g)	91.74	114.94
$q_{e,exp}$ (mg/g)	91.44	110.87
R^2	0.9870	0.9966
Intraparticle diffusion		
k_d	3.2017	3.9637
C	33.07	46.34
R^2	0.9519	0.9041

a: The pseudo-first-order model: $\lg(q_e - q_t) = \lg q_e - {}^{k_1}\!/_{2.303} t$, pseudo-second-order model: ${}^{t}\!/_{q_t} = {}^{1}\!/_{k_2 q_e{}^2} + {}^{1}\!/_{q_e} t$ and intraparticle diffusion model: $q_t = k_d t^{1/2} + C$

Table 4 Kinetic parameters of stages with intraparticle diffusion model for co-pellets at optimum adsorption conditions.

	Live co-pellets			Lyophilized co-pellets		
Stage	k_3 mg/(g▪min$^{1/2}$)	C	R^2	k_3 mg/(g▪min$^{1/2}$)	C	R^2
I	6.46	18.46	0.9557	7.85	26.44	0.9990
II	2.48	43.52	0.9739	4.64	46.00	0.9767
III	-	-	-	0.61	99.61	0.8415

Table 5 Comparison of Langmuir, Freundlich and Temkin adsorption isotherm[a] constants for co-pellets at optimum adsorption conditions.

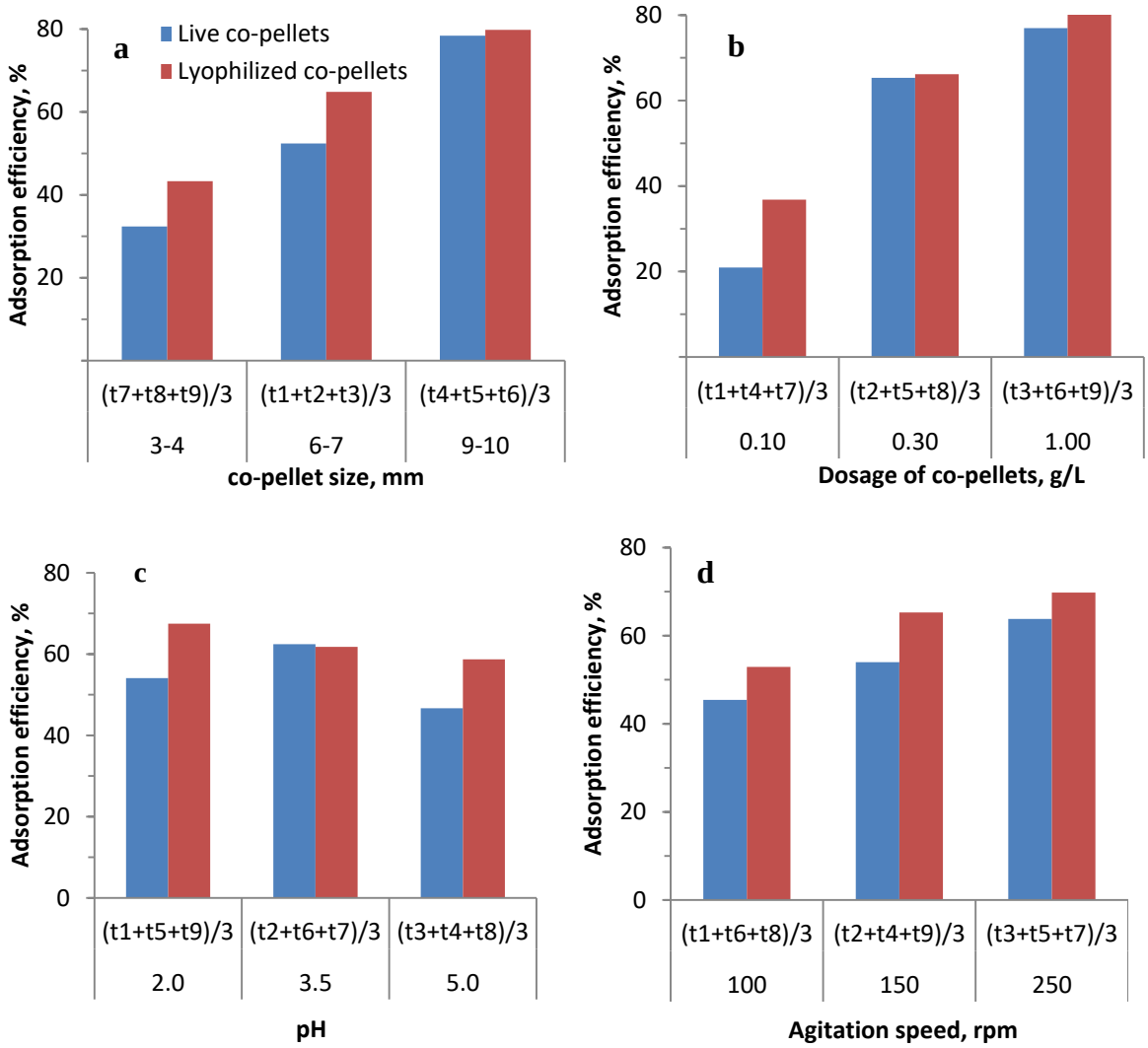
Parameters	Live co-pellets	Lyophilized co-pellets
Langmuir constant		
q_m (mg/g)	104.17	112.36
b (L/mg)	0.15	0.41
R^2	0.9971	0.9983
Freundlich constant		
K_f	52.94	78.69
n	7.54	13.28
R^2	0.9498	0.8997
Temkin constant		
A (L/g)	61.52	61148
B	222.78	350.12
R^2	0.9419	0.8753

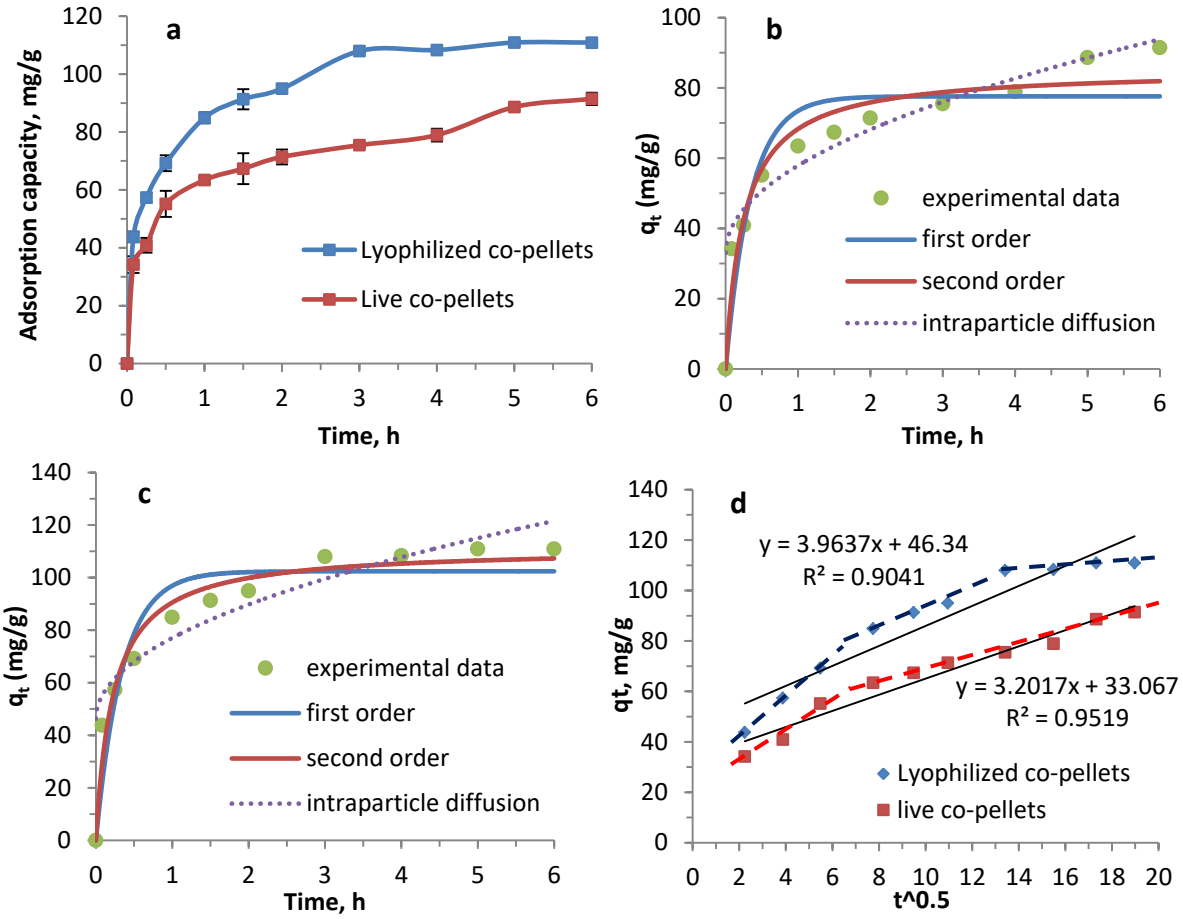
a: The Langmuir adsorption model: $C_e/q_e = C_e/q_m + 1/bq_m$; Freundlich model: $\ln q_e = \ln K_f + 1/n \ln C$ and Temkin model : $q_e = B\ln A + B\ln Ce$.

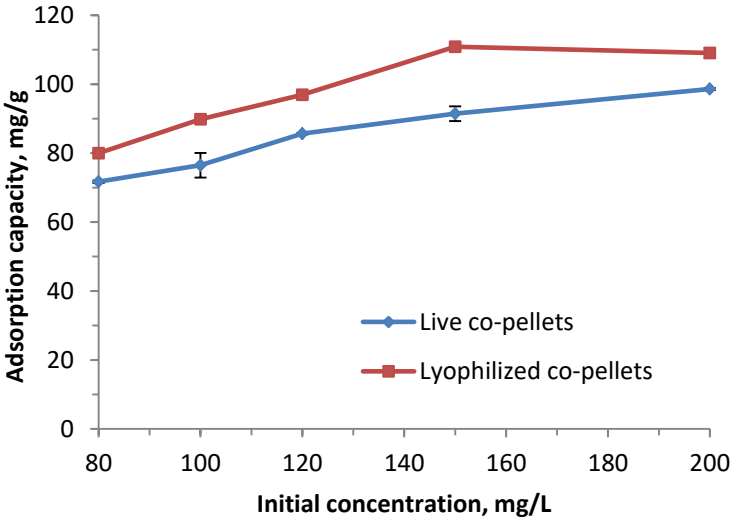
Table 6 The components and concentrations in 1L wastewater before and after adsorption by 9-10mm lyophilized co-pellets in SBR (pH=2.0, 25±2 °C, 60 min).

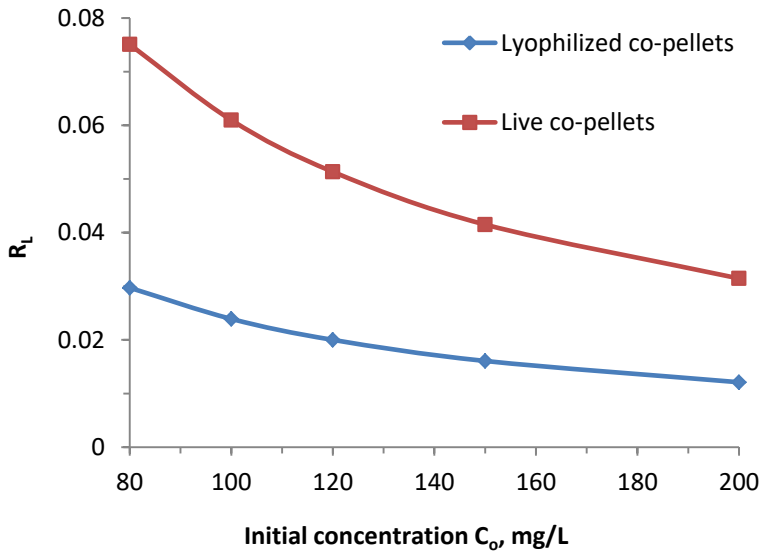
Elements	Lab wastewater			Gold-mining wastewater[a]			PCB wastewater		
	Before mg/L	After mg/L	%[b]	Before mg/L	After mg/L	%[b]	Before mg/L	After mg/L	%[b]
Na	484.9	415.2	14.17	291.4	306	0	5.32	11.15	0
Mg	3.7	5.7	0	3.4	5.1	0	1.9	2.4	0
K	33.4	46.2	0	57.7	75.8	0	354.4	375	0
Au	68.4	46.27	**32.35**	30	13.61	**54.63**	30	0.67	**97.77**
Ca	13	16.4	0	325.2	250.8	22.88	1.7	4.74	0
Ni	0.1	0.1	0	0.9	0.9	0	15.8	13.61	13.86
Co	<0.02	<0.02	0	0.5	0.4	20	2.1	1.84	12.38
Cu	1.5	1.4	6.67	1.5	0.79	47.33	0.06	0.07	0
Hg	0.08	0.08	0	1.3	0.7	46.15	0.02	0.06	0
CN-	<0.01	<0.01	0	17	14	17.65	0	0	0
Thiourea[c]	2.19	0.43	80.37	0	0	0	0	0	0

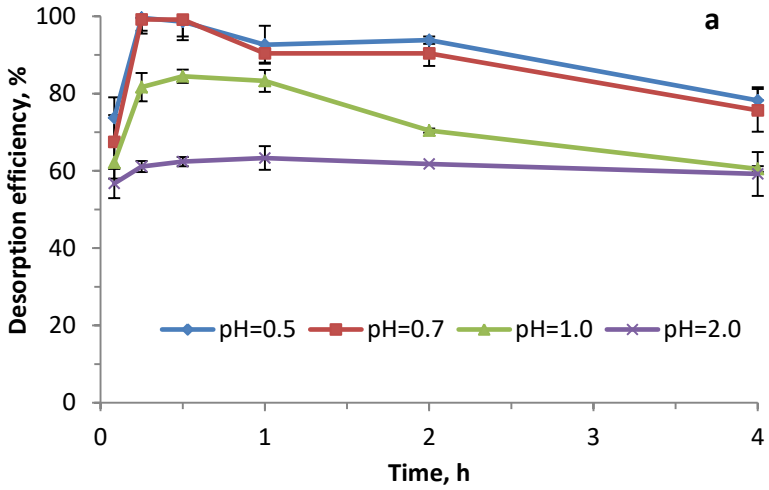
a: the original gold concentration in wastewater was negligible with only 2 mg/L and modified to 30 mg/L by adding $HAuCl_4$; b: the average adsorption efficiency of three samples from different outlets on SBR; c:thiourea concentration, M.

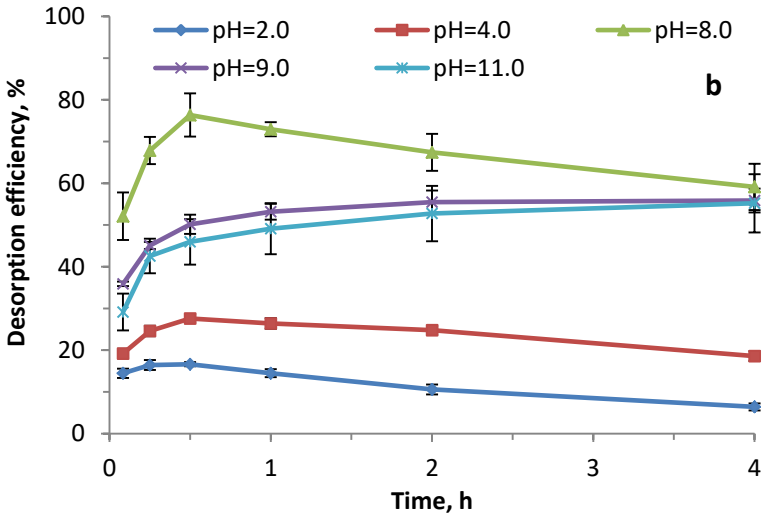

a
Live co-pellets
Lyophilized co-pellets
Adsorption efficiency, %
80
60
40
20
(t7+t8+t9)/3
3-4
(t1+t2+t3)/3
6-7
(t4+t5+t6)/3
9-10
co-pellet size, mm
b
Adsorption efficiency, %
(t1+t4+t7)/3
0.10
(t2+t5+t8)/3
0.30
(t3+t6+t9)/3
1.00
Dosage of co-pellets, g/L
c
Adsorption efficiency, %
0
(t1+t5+t9)/3
2.0
(t2+t6+t7)/3
3.5
(t3+t4+t8)/3
5.0
pH
d
Adsorption efficiency, %
(t1+t6+t8)/3
100
(t2+t4+t9)/3
150
(t3+t5+t7)/3
250
Agitation speed, rpm

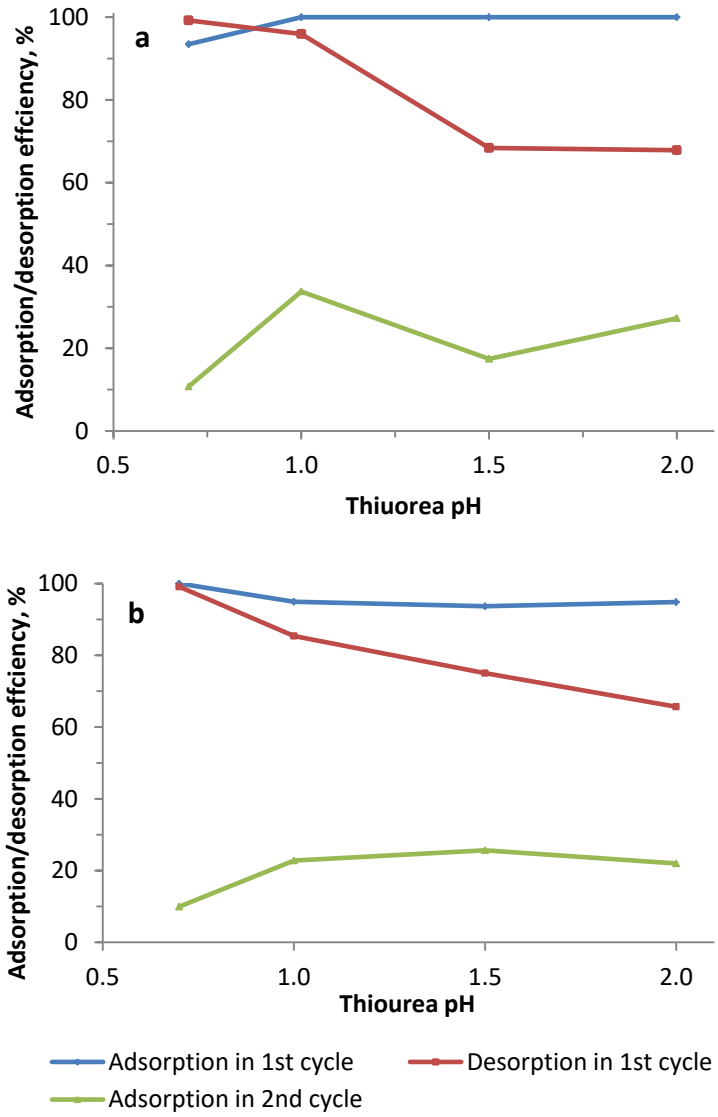

a
Adsorption capacity, mg/g
Time, h
Lyophilized co-pellets
Live co-pellets
b
q_t (mg/g)
Time, h
experimental data
first order
second order
intraparticle diffusion
c
q_t (mg/g)
Time, h
experimental data
first order
second order
intraparticle diffusion
d
qt, mg/g
t^0.5
y = 3.9637x + 46.34
R² = 0.9041
y = 3.2017x + 33.067
R² = 0.9519
Lyophilized co-pellets
live co-pellets


120
100
80
60
40
20
0
Adsorption capacity, mg/g
80
100
120
140
160
180
200
Initial concentration, mg/L
Live co-pellets
Lyophilized co-pellets


0.08
0.06
0.04
0.02
0
R_L
80
100
120
140
160
180
200
Initial concentration C_o, mg/L
Lyophilized co-pellets
Live co-pellets


a
Desorption efficiency, %
100
80
60
40
20
0
0
1
2
3
4
Time, h
pH=0.5
pH=0.7
pH=1.0
pH=2.0


b
Desorption efficiency, %
100
80
60
40
20
0
0
1
2
3
4
Time, h
pH=2.0
pH=4.0
pH=8.0
pH=9.0
pH=11.0


a
Adsorption/desorption effciency, %
100
80
60
40
20
0
0.5
1.0
1.5
2.0
Thiuorea pH
b
Adsorption/desorption effciency, %
100
80
60
40
20
0
0.5
1.0
1.5
2.0
Thiourea pH
Adsorption in 1st cycle
Desorption in 1st cycle
Adsorption in 2nd cycle