The use of activated sludge and dried sludge for the removal of Cd(II) ions from aqueous solutions

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ABSTRACT

This research presented the removal of Cd(II) from aqueous solutions using two biosorbents: activated sludge (AS) and dried sludge (DS), derived from two cultures differing in medium composition. Cadmium adsorption proceeded at a very high rate. Adsorption equilibrium was reached after ca. 10 min. The amount of adsorbed metal ions was influenced by solution pH, contact time, and initial concentration of cadmium. The adsorption capacity of the analyzed sludge increased along with a pH increase from 2 to 6. The highest adsorption capacity was obtained for dried sludge from culture 2 (DS2) at pH > 6. Experimental data concerning adsorption at the equilibrium state was analyzed using isotherms of Freundlich, Langmuir, and Sips models. The best model to describe Cd(II) desorption on biosorbents turned out to be the Sips model, which was indicated by values of the average relative error. A higher adsorption capacity was obtained for dried sludge (DS2 – 185.6 mg/g d.m.) than for the activated sludge (AS1 and AS2 – 141.7 and 164.1 mg/g d.m., respectively). The study demonstrated that DS2 sludge may be a promising biosorbent in effective and inexpensive removal of toxic cadmium from wastewater.

Keywords: Cadmium ions; Biosorption; Activated and dried sludge; Water treatment; Triphenyl tetrazolium chloride

1. Introduction

For many years, cadmium has been on various lists of especially hazardous chemical compounds due to its toxicity and capability to accumulate in plant and animal organisms [1,2].

Its presence in the natural environment is due to both natural processes, like erosion of rocks or volcanic eruptions, as well as anthropogenic ones associated with material extraction, production of dyes, batteries, paints, and plastics [3–6]. A serious source of surface waters contamination with cadmium is wastewater from the electroplating industry, from dye production and from the production of nickel-cadmium batteries. Due to the high toxicity of cadmium,

it is necessary to adhere to guidelines concerning its concentration in drinking water. According to WHO guidelines, the maximum permissible cadmium concentration in drinking water is 0.005 mg/L. Therefore, a need for using such processes that would enable the recovery of heavy metals from waste, sewage or waters to minimize their negative effects on living organisms has been created.

Conventional methods used to remove heavy metals, such as chemical precipitation, filtration, ion exchange or reverse osmosis, despite their high efficiency, are often uneconomical, especially in the case of low concentrations of metal ions [7]. Because of that, methods that are cheaper, more effective and safe for the environment,

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using materials of biological origin (biosorbents) are being searched for.

Biosorption has been deemed a promising and inexpensive technology that allows removing even trace amounts of heavy metals from solutions [8,9].

Agricultural waste such as fruit peels, straw, coconut coin, grape pomace, rice husk and similar [10–13], as well as industrial waste, such as sugarcane bagasse, coconut tree sawdust [7,14], various types of modified, activated carbon and biochar [15–16] and microorganisms such as bacteria, yeasts, fungi and algae [17–21] were all used as biosorbents for the removal of heavy metals.

Biosorbents from agricultural waste are easily obtained by a simple pretreatment process and often have specific functional groups such as –OH or –COOH, which can generate an adsorption affinity for heavy metal ions [7].

One of the effective biosorbents is the biomass of microorganisms being a by-product in processes of wastewater treatment using activated sludge [22–24]. From the microbiological point of view, activated sludge is mainly composed of bacteria and protozoa with high species diversity of its microorganisms.

Live biomass yield depends on the availability of nutrients, microorganism's resistance to toxicity, and age of cells. The general biochemical activity of sludge is usually assessed based on its respiratory activity, measured with the rate of oxygen consumption by sludge microorganisms and the activity of its dehydrogenases [25,26]. The latter are enzymes from the class of oxydoreductases which catalyze the initial dehydrogenation of a substrate. Dehydrogenase activity determination is based on the reduction of biologically active substances of tetrazolium salts to triphenyl formazan [27]. The tetrazolium salts play a role of a hydrogen acceptor during redox processes. They turn from colorless substances into colored triphenyl formazan.

A drawback of biosorbents in the form of free biomass is their low mechanical resistance and their difficult separation from the solution. The use of dead biomass eliminates these problems and additionally its deposition, regeneration, and re-use are significantly easier [28].

This study aimed at evaluating the effect of medium composition in the culture of activated sludge biomass and of biomass type (active/dead) on the effectiveness of cadmium removal from aqueous solutions and at determining the effect of activated sludge dehydrogenase activity on an increasing cadmium concentration. There is a need for more insightful recognition of mechanisms of metal removal from the solution, for determining operational conditions of this process including pH value and time needed to reach equilibrium concentration, and for determining the adsorption capacity of biosorbents.

2. Methods

2.1. Sludge culture

Activated sludge from the biochemical wastewater treatment plant in Olsztynek was used as the inoculum.

The culture was run in a sequential batch reactor (SBR) type reactor (Fig. 1) with a volume of 20 L.

Wastewater was fed to the reactor once a day, after prior sedimentation of activated sludge and decantation of water from above the sludge. Excess sludge was withdrawn once a day to maintain biomass concentration in the reactor at 4 g/L.

Medium 1 used for sludge culture was composed of: enriched broth – 0.15 g, peptone – 0.05 g, potato starch – 0.05 g, urea – 0.3 g, CH_3COONa – 0.1 g, $CaCl_2 \cdot H_2O$ – 0.07 g, $MgSO_4 \cdot 7H_2O$ – 0.5 g, NaCl – 0.3 g, and KCl – 0.07 g/1 L. The content of organic matter in the culture medium expressed as COD value reached 660 mg O_2/L, Kjeldahl nitrogen – 99.4 mg N_{og}/L, total phosphorus – 14.4 mg P_{og}/L, and pH was 7.29.

Medium 2 used for sludge culture was composed of: TRIS buffer – 12 g, $(NH_4)_2SO_4$ – 0.96 g, 2-phosphate glycerol (disodium salt hydrate, $5H_2O$) – 0.67 g, KCl – 0.62 g, $MgSO_4 \cdot 7H_2O$ – 0.06 g, glycerin – 0.06 g, and $FeSO_4$ – 3.2 g/1 L [29]. The content of organic matter in the culture medium expressed as COD value reached 3720 mg O_2/L, Kjeldahl nitrogen – 498.96 mg N_{og}/L, total phosphorus –34 mg P_{og}/L, and pH was 8.97.

2.1.1. Sludge preparation for analyses

2.1.1.1. Activated sludge

Activated sludge collected from culture 1 (AS1) and culture 2 (AS2) was centrifuged at 4,000 rpm for 15 min.
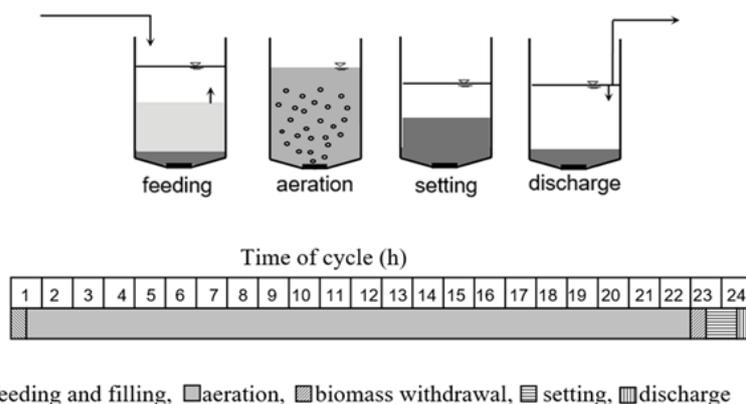

Fig. 1. The work cycle of SBR reactor.

The centrifuged sludge was washed with distilled water and centrifuged again to wash out nutrients.

2.1.1.2. Dried sludge

Activated sludge collected from culture 1 (DS1) and culture 2 (DS2) was centrifuged at 4,000 rpm for 15 min. The centrifuged sludge was washed twice with acetone, centrifuged again, and left in water bath at a temperature of 50°C for 48 h. The resultant dried sludge was ground and sieved through a screen with mesh size of 0.01 mm.

2.2. Determination of the effect of cadmium concentration on the activity of dehydrogenases of sludge microorganisms

The effect of cadmium concentration on the activity of microorganism dehydrogenases of active sludge AS1 and AS2 were determined at following concentrations: 0, 5, 50, and 150 mg Cd/L. Samples of sludge were collected for analysis after 1, 5, 10, 15, and 30 min, and the activity of dehydrogenases was determined using a solution of 1% TTC (2,3,5-triphenyltetrazolium chloride) with 2% glucose as a substrate, after 0.5-h incubation at a temperature of 37°C and extraction of the produced TF (2,3,5-triphenyltetrazolium formazan) with n-butanol. Color intensity was measured with a spectrophotometer at a wavelength of 490 nm.

2.3. Sorption analysis

2.3.1. Effect of pH

The analysis was carried out in a pH range from 1 to 10, and Cd concentration of 100 mg/L. The biosorbents (AS1, AD1, DS1, and DS2) in the amount of 0.4 g d.m. each was placed into a 250 mL reaction vessel, then 100 mL of a cadmium solution (with pH adjusted to the desired value using 0.01 M NaOH or 0.01 M HNO$_3$) was added to each mixture and shaken at 180 rpm for 2 h.

2.3.2. Effect of time

The analysis of cadmium adsorption by the tested biosorbents included the determination of the time needed to reach the reaction equilibrium. To analyze that, 2 g d.m. of each biosorbent was weighed into a reaction vessel and 500 mL of Cd(II) solution in concentrations ranging from 5 to 150 mg/L were added to the vessels. The vessels were placed on a magnetic stirrer. Samples were collected in a time interval of 0–30 min and the concentration of cadmium left in the solution was determined.

2.3.3. Adsorption capacity

Concentrations of Cd(II) in the solutions were adjusted so as to achieve the course of adsorption isotherm until complete saturation of active sites of biosorbents with cadmium. Standard solutions were prepared in concentrations ranging from 1 to 600 mg/L., where 0.4 g d.m. of each biosorbent and 100 mL of metal solution with a specified concentration were introduced into 250 mL reaction flasks. The flasks were then shaken at 180 rpm.

2.4. Analytical method

For AAS, analytical grade standard solutions of Cd (1.000 g/L) obtained from Sigma-Aldrich were used as stock solutions. All working solutions were prepared by diluting the stock solutions with deionized water. Solutions of 0.1 M HNO$_3$ and 0.1 M NaOH were used for pH adjustment. The concentration of metals left in the aqueous solution of each sample was checked with the flame method using an AA 280FS atomic adsorption spectrometer by Varian. The measurements of metal concentration were performed at the wavelengths of 228.8 nm. Oxidant (air) and fuel (acetylene) flows were 13.5 and 1.5 L/min, respectively. Absorbance was recorded in triplicate to evaluate the reproducibility. Mean values were used for the concentration calculation (average error did not exceed 5%). The blank experiments were always carried out for each study without biosorbent to check any deviation in the solute concentration.

Identification of functional groups and the type of the bonds was performed by Fourier-transform infrared spectroscopy (FTIR) with a spectrometer with a mono-reflective ATR attachment. Before FTIR analysis, the sorbent was dehydrated in a hydraulic press. A dehydrated sample in compressed form was placed on the diamond crystal of the ATR attachment and then pressed down to the crystal with the "pressure screw". The adsorption of the sample was determined at wavelengths 400–6,000 cm^{-1}, with a resolution of 2 cm^{-1}. Each sample was scanned 64 times, and then the mean value was calculated. The ATR crystal attachment was cleaned with acetone between the analyses of different sorbents.

3. Theory

The quantity of metal adsorbed from the solution was determined based on the change in the concentration of metal left in the solution, and calculated according to the following formula:

$$Q_s = \frac{C_0 - C_s}{m} \cdot V \tag{1}$$

where Q_s is the mass of metal adsorbed by the biosorbent (mg/g d.m.), C_0 is the initial concentration of metal in the solution (mg/L), C_s is the metal concentration in the solution after adsorption (mg/L), m is the weight of biosorbent (g d.m.), and V is the volume of metal-containing solution (L).

The rate of cadmium adsorption was determined based on experimental data depicting changes in the concentration of adsorbed Cd(II) (q_t) depending on time (t). The pseudo-second-order reaction model was used to interpret the results:

$$\frac{dq_t}{dt} = k\left(q_e - q_t\right)^2 \tag{2}$$

where q_e and q_t (mg/g d.m.) are the amount of metal adsorbed at equilibrium and at time t (min), respectively, and k is the pseudo-second-order rate constant (g/mg min).

To fit the equilibrium adsorption data, the Langmuir, Freundlich and Sips isotherm equations were examined. They are given, respectively, by Eqs. (3–5):

$$Q_{s,d} = \frac{q_{max} \cdot b \cdot C_s}{1 + b \cdot C_s} \tag{3}$$

$$Q_{s,d} = K_F C_s^{1/n} \tag{4}$$

$$Q_{s,d} = \frac{q_{max} \cdot b \cdot C_s^{1/n}}{1 + b \cdot C_s^{1/n}} \tag{5}$$

where q_{max}, b, K_F, n, are constants of isotherms and C_s are metal concentration in the solution after adsorption/desorption, Q_s mass of metal adsorbed/desorbed by/from the biosorbent.

STATISTICA 10.0 software was used to determine the fitting of the curves (with the determined constant) to the experimental data with the use of non-linear estimation by the method of least squares, at a significance level of $p < 0.05$.

The minimization of the average relative error (ARE) was computed as follows in order to show how well the equilibrium models agreed with experimental results [30]:

$$ARE(\%) = \frac{100}{z} \sum_{i=1}^{z} \left(\frac{|q_{exp} - q_{calc}|}{q_{exp}} \right)_i \tag{6}$$

where z is the number of data points; q_{exp} and q_{calc} are the experimental adsorption capacity and the adsorption capacity calculated with the theoretical models.

4. Results and discussion

4.1. Effect of cadmium concentration on the activity of dehydrogenases of activated sludge microorganisms

The study demonstrated that AS1 and AS2 biosorbents were biochemically active, which was indicated by the results of the dehydrogenase activity test (TTC). The analysis of the activities of microorganisms determined in two cultures showed that AS1 had a higher activity (7.0 mg TF/g d.m.) than AS2 (4.0 mg TF/g d.m.). Hence, it may be concluded that the activity of sludge microorganisms depended on the type of sludge culture.

In this research, we evaluated the effect of an increasing cadmium concentration (from 50 to 150 mg /L) on the activity of dehydrogenases of activated sludge microorganisms. Analysis of the toxic effect of cadmium on the enzymatic activity of these microorganisms demonstrated that metal introduction into the activated sludge suppressed the activity of dehydrogenases in sludge biomass. The greatest decrease in the biochemical activity of AS1 and AS2 was noted in the sample with the initial cadmium concentration of $C_0 = 150$ mg /L, where a decrease by 43.5% and 82.6%, respectively, was observed in the TTC value already after 1 min compared to the control sample (Fig. 2a and b).

Equally toxic effects of nickel on the biochemical activity of activated sludge microorganisms cultured in a SBR were demonstrated by Ong et al. [31]. A significant inhibiting effect of nickel on the rate of oxygen uptake by microorganisms of the activated sludge was noted for Ni(II) concentration over 30 mg/L. At nickel concentration of 150 mg/L, the activity of microorganisms was suppressed by over 50%. Investigations on the effect of two different concentrations of cadmium and copper on the activity of activated sludge dehydrogenases [32] showed that even the lowest concentrations (0.5–8.0 mg/L) of their ions caused significant changes in sludge dehydrogenase activity. Ions of copper were more toxic than these of cadmium.

4.2. FTIR analysis of biosorbents

Biosorption of cadmium ions by the biomass of activated sludge is largely dependent on functional groups present at active sites of cells of sludge microorganisms and on physicochemical conditions of the solution. For better recognition of which functional groups are involved in the biosorption process, the biomass of the sludge was subjected to FTIR analysis (Fig. 3). Spectra obtained using FTIR spectroscopy for both AS1 and AS2 (Fig. 3a and c)
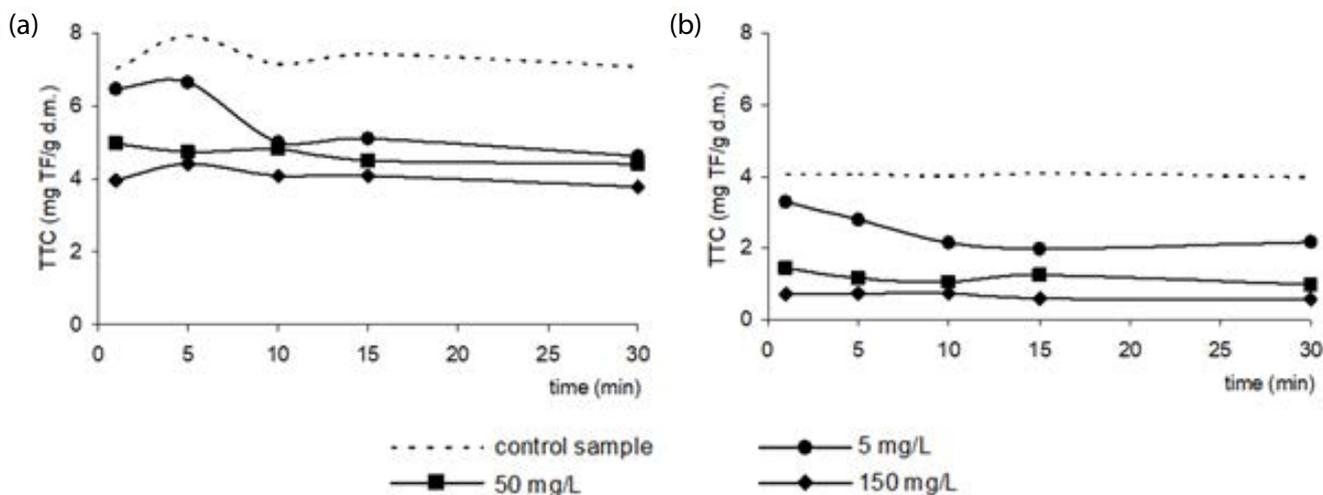

Fig. 2. Activity of dehydrogenases of activated sludge (TTC): (a) AS1 and (b) AS2.
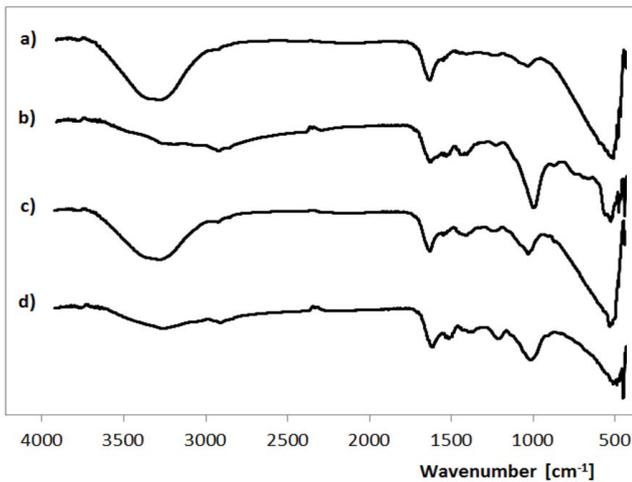
Fig. 3. FTIR spectrum: (a) AS1, (b) DS1, (c) AS2, and (d) DS2.

show similar peaks being typical for a number of functional groups occurring in activated sludges. In case of both activated sludges, a wide adsorption band appears at 3,800–3,200 cm^{-1} as a result of oscillations of –OH and –NH, which is indicative of the presence of water and amines.

Small peaks noted for both type of sludge cultures (active/dead) appear at ca. 2,920 cm^{-1} and may be caused by oscillations of –CH indicating the presence of aliphatic chains. The band appearing at 1,640 cm^{-1} in spectra of all analyzed sludges confirms the presence of proteins and is, probably, induced by the stretching oscillations of COO, C=O, and C–N [30,33]. A peak at 1,443 cm^{-1} depicts oscillations of C–O and O–H groups originating from phenolic compounds [34,35]. In regard to sludges from culture 2 (AS2/DS2), peaks appear in the FTIR spectrum at ca. 1,240–1,243 cm^{-1} (Fig. 3c and d). According to literature data, these peaks may indicate the presence of amides III, in case of which oscillations appear at 1,300–1,220 cm^{-1} [36]. In both spectra obtained for culture 1(AS1/DS1), a band appears at 995 cm^{-1}, which depicts oscillations of C–O [37], whereas in both spectra obtained for culture 2 (AS2/DS2) a band appears at 1,028 cm^{-1}, which – according to literature data – is indicative of P–O oscillations in terminal PO$_3^{2-}$ [38]. The other peaks appearing at <1,000 cm^{-1} for both cultures (AS1/DS1/AS2/DS2) are probably indicative of the presence of functional groups containing phosphorus and sulfur [39].

Considering that a peak appeared in spectra obtained for culture 2 (Fig. 3c and d) which did not appear in spectra of culture 1 (Fig. 3a and b), it may be concluded that the effectiveness of cadmium sorption differed. The peak at 1,028 cm^{-1} obtained for AS2 and DS2 sludge was probably appearing as a result of glycerol 2-phosphate presence in the culture medium which was a source of phosphorus compounds retained in cell surface.

4.3. Effect of solution pH on cadmium adsorption

As indicated by literature data, each system consisting of an aqueous solution is characterized by pH that enables maximum adsorption of metals by the adsorbent [40,41].

Sludge surface is negatively charged, which facilitates its binding with positively charged metal cations. Nevertheless, depending on solution pH, cations of metals and protons may compete for active sites of the biomass. The effect of solution pH on cadmium biosorption by the analyzed biosorbents is depicted in Fig. 4.

The lowest adsorption of cadmium was determined at pH = 2.0 (4.5 mg/g d.m. on average), which could be due to protonation of adsorption sites and, as a result, by desorption of originally bound metal. A similar trend of cadmium adsorption was noted for all analyzed sludges at solution pH of 2–6 when a successive increase was observed in metal capture that might be explained by the fact that solubility of many metals decreases along with pH increase, which enhances sorption. A further increase in solution pH did not increase Cd(II) adsorption by the sludge from culture 2 (AS2 and DS2). In the case of sludge from culture 1 (AS1 and DS1), pH increase from 7 to 9 diminished cadmium adsorption to the value of 6.3 and 9.6 mg/g d.m., respectively, and this decrease could be due to reduced solubility of cadmium in the basic medium and to its precipitation. Similar results of pH effect on cadmium adsorption were reported by other authors [42–44]. However, in the research of Iqbal at all. [45] it is shown that the lower removal efficiency of As^{3+} by AC/nZVCu/HA-alginate beads and greater mobility of H$_3$O$^+$ in the aqueous solution that consequently compete with As^{3+} was at pH < 5.0 and pH > 7.0. In my own research a higher adsorption capacity at pH = 6 was determined for AS2 and DS2, that is, 22.4 and 24.2 mg/g d.m., respectively. Simultaneously, a higher adsorption capacity was observed for dried sludge DS1 and DS2 (Fig. 4).

4.4. Analysis of adsorption kinetics

Determination of biosorbent usefulness for cadmium removal from wastewater by adsorption requires knowing the rate of this process. In this study, the time needed to reach reaction equilibrium was established for initial cadmium concentration ranging from 5 to 150 mg/L (Fig. 5). The kinetics of cadmium biosorption by AS1 and AS2 showed a rapid increase of cadmium content in the biosorbent in the first minutes of contact (Fig. 5a and c) which was followed by a slow increase until the
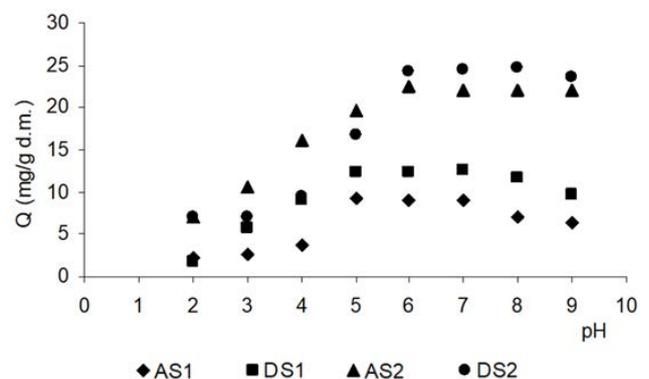

Fig. 4. Effect of solution pH on the efficiency of Cd(II) adsorption (C_0 = 100 mg/L, Temp. 25°C).

(a)

(b)

(c)

(d)

—————— pseudo-second order model

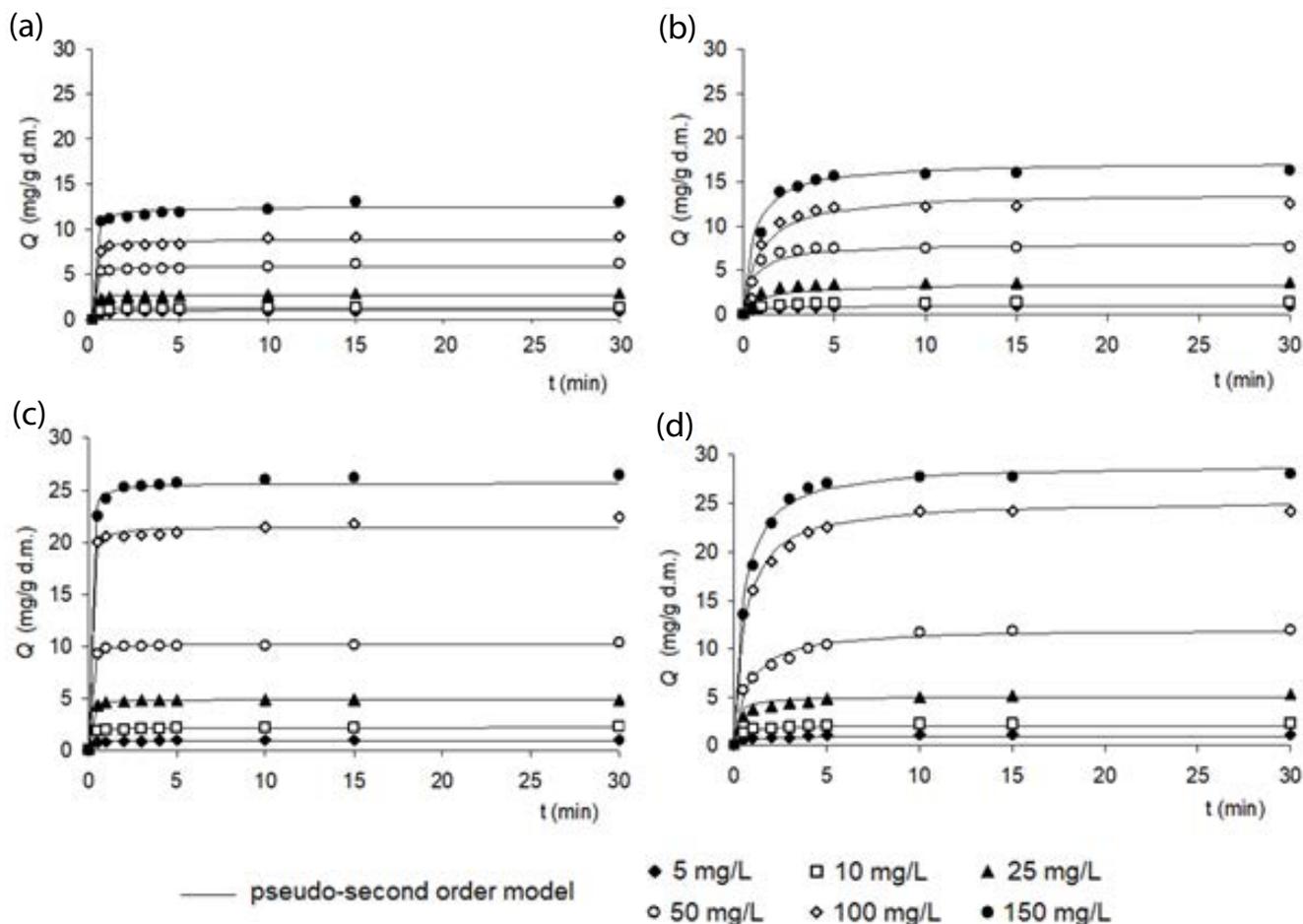◆ 5 mg/L   □ 10 mg/L   ▲ 25 mg/L
○ 50 mg/L   ◇ 100 mg/L   ● 150 mg/L

Fig. 5. Correlation between the mass of adsorbed metal and time of adsorption reaction: (a) AS1, (b) DS1, (c) AS2, and (d) DS2 (pH = 6 ± 0.5, Temp. 25°C).

equilibrium state has been achieved. The time required to reach this state reached ca. 10 min, and its extension to 30 min had no significant effect on cadmium content in the biosorbent. Adsorption efficiency in the equilibrium state depended on the initial concentration of cadmium and culture type. At the initial cadmium concentration of $C_0 = 5$ mg/L, the effectiveness of metal adsorption by activated sludge (AS1 and AS2) was comparable and reached 76% and 78%, respectively. In contrast, significant differences were noted at $C_0 = 150$ mg/L (AS1 – 35%, AS2 – 71%). In the case of dried sludge (DS1 and DS2), reaction equilibrium was achieved after 15 min and significant differences in cadmium adsorption effectiveness were noted in the entire analyzed range of initial cadmium concentrations. At the equilibrium state, 58% of the initial Cd concentration was removed from the solution by DS1 and 89% by DS2 (Fig. 5b and d). It may be concluded that the effectiveness of cadmium removal was high at its low initial concentrations and decreased along with the rise in concentrations, which might be associated with a change in the composition of sludge biomass and in the structure of floccules [31].

To describe the experimental data, a pseudo-second-order model was used [46–48]. Determination of kinetic constants

enabled establishing the rate of reaching equilibrium conditions (k) and mass of cadmium (Q) adsorbed under these conditions. The analysis of kinetic constants (Q) determined form the pseudo-second-order model showed the dependency of cadmium adsorption on its initial concentration and sludge type (Fig. 6).

In case of DS2, the Q values obtained for cadmium concentration of 150 mg/L were over 30 times higher compared to these obtained for cadmium concentration of 5 mg/dm³ and reached 29.1 and 0.94 mg/g d.m., respectively. A similar correlation was observed for the remaining bisorbents. In addition, increased dried sludge uptake at high initial concentrations of cadmium was observed compared to activated sludge, which could be due to the toxic effect of cadmium on the adsorption mechanism of activate sludge microorganisms [49].

In case of lower initial concentrations of cadmium, its adsorption was faster due to the adsorption surface. At a high number of available adsorption sites, the rate of the reaction was higher and this dependency was noted for all analyzed biosorbents. The highest values of k constant were observed for activated sludge at initial cadmium concentration of 5 mg/L, that is, 4.32 g/mg min for AS1 and 5.07 g/mg min for AS2. A similar dependency of adsorption rate decrease along
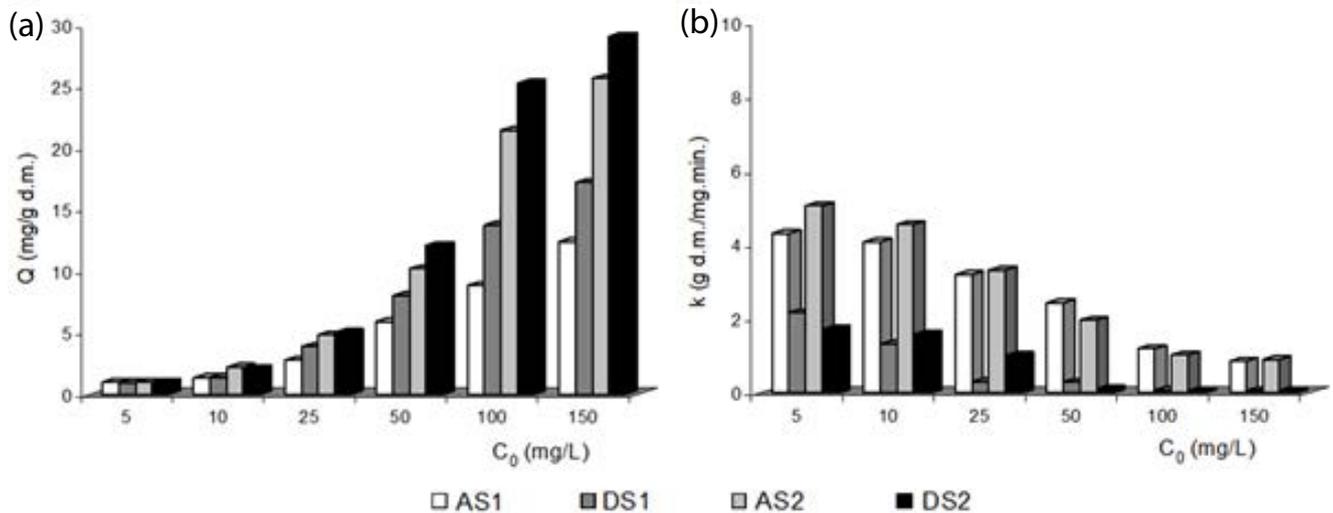
Fig. 6. Constants determined from the pseudo-second-order equation for the analyzed biosorbents: (a) Q (mg/g d.m.) and (b) k (g d.m./mg min).

with metal concentration increase was demonstrated by Benaïssa and Elouchdi [50] in their study on Cu(II) removal by dried sludge from a biological wastewater treatment plant.

4.5. Determination of the maximum adsorption capacity

From the economic point of view, a sorbent should exhibit both good adsorption properties (i.e., be characterized by a high adsorption capacity) and a high recovery of adsorbed substances. To compare the capability of various biosorbents for the adsorption of metals and their affinity to metals, the adsorption process may be expressed as an isotherm in the equilibrium state determined under static conditions. Adsorption may be modeled using theoretical equations which may explain or predict the course of the process under experimental conditions or using empirical equations which may reflect experimental curves but do not describe the mechanism of biosorption per se. The most common models used in such analyses are Langmuir and Freundlich's models owing to their reliability and simplicity, and to the easiness of data interpretation.

In our study, biomass sorption capabilities were evaluated using Langmuir, Freundlich, and Sips models. Experiments were conducted at the initial solution pH of 6.0, temp. of 25°C, and varying initial concentrations of Cd(II) ions: from 1 to 600 mg/L. Experimental results concerning the amount of adsorbed Cd(II) depending on metal concentration left in the solution as well as Langmuir, Freundlich, and Sips isotherms determined on their basis are depicted in Fig. 7. It was concluded that isotherms of the applied adsorption models fitted well to experimental data of cadmium adsorption by the tested biosorbents, except for the Freundlich isotherm.

To enable further results analysis of Cd(II) adsorption on tested biosorbents, values of constants were determined from Eqs. (3)–(5), determination coefficient R^2, and ARE are presented in Table 1. Based on R^2 values it was difficult to assess the usability of adsorption models equations for the description of experimental data. Values of this coefficient

were very high (0.9970–0.9988) for isotherms calculated from Langmuir and Sips models and significantly lower (0.9474–0.9661) in case of the Freundlich model (Table 1). The ARE turned out to be significantly more useful. Its values were more diversified, which allowed to choose the model that described the experimental data of cadmium adsorption best. The study demonstrated, that the Sips model was the most suitable for the description of Cd(II) adsorption for both active and dead sludges, which was indicated by the determined ARE values (Table 1).

The Freundlich model appeared to be the least suitable for the description of the process, which was indicated by the highest ARE values. In a study on the adsorption of Cd^{2+}, Cu^{2+} and Pb^{2+} on alginate obtained from *Laminaria digitata* algae, Papageorgiou et al. [51] demonstrated that isotherms determined from both Langmuir and Sips models described better the mechanisms of adsorption of the tested metals compared to Freundlich isotherm. The efficiency and versatility of the Sips model were also confirmed by Nagy et al. [52] who investigated the adsorption of Cd^{2+} and Zn^{2+} ions from an aqueous solution on *Agaricus bisporus* biomass. They analyzed their experimental data using: Langmuir, Freundlich Dubinin-Radushkevich, Temkin, Redlich-Peterson, Sips, Toto, Khan, and Radke-Prausnitz models at pH 5.2 (Cd) and 5.6 (Zn) and biosorbent dose of 4 g/L. They also showed the best fit to experimental data for Sips isotherm.

Data presented in Table 1 indicated that the analyzed biosorbents were characterized by both diversified sorption capacity and affinity to metals. Dried sludge showed a higher adsorption capacity compared to activated sludge. In addition, inactivation of biomass cells caused an over 2-fold and 4-fold increase in the value of b constant in case of DS1 and DS2, respectively. In their study on Cd(II) adsorption by cells of *Pseudomonas aeruginosa* (PU21), Chang et al. [53] reported an almost 20-fold increase in b constant determined from Langmuir model to the value of 1.25 L/mg for dead cells and a decrease in the maximum sorption capacity q_{max} by ca. 30% compared to active cells.
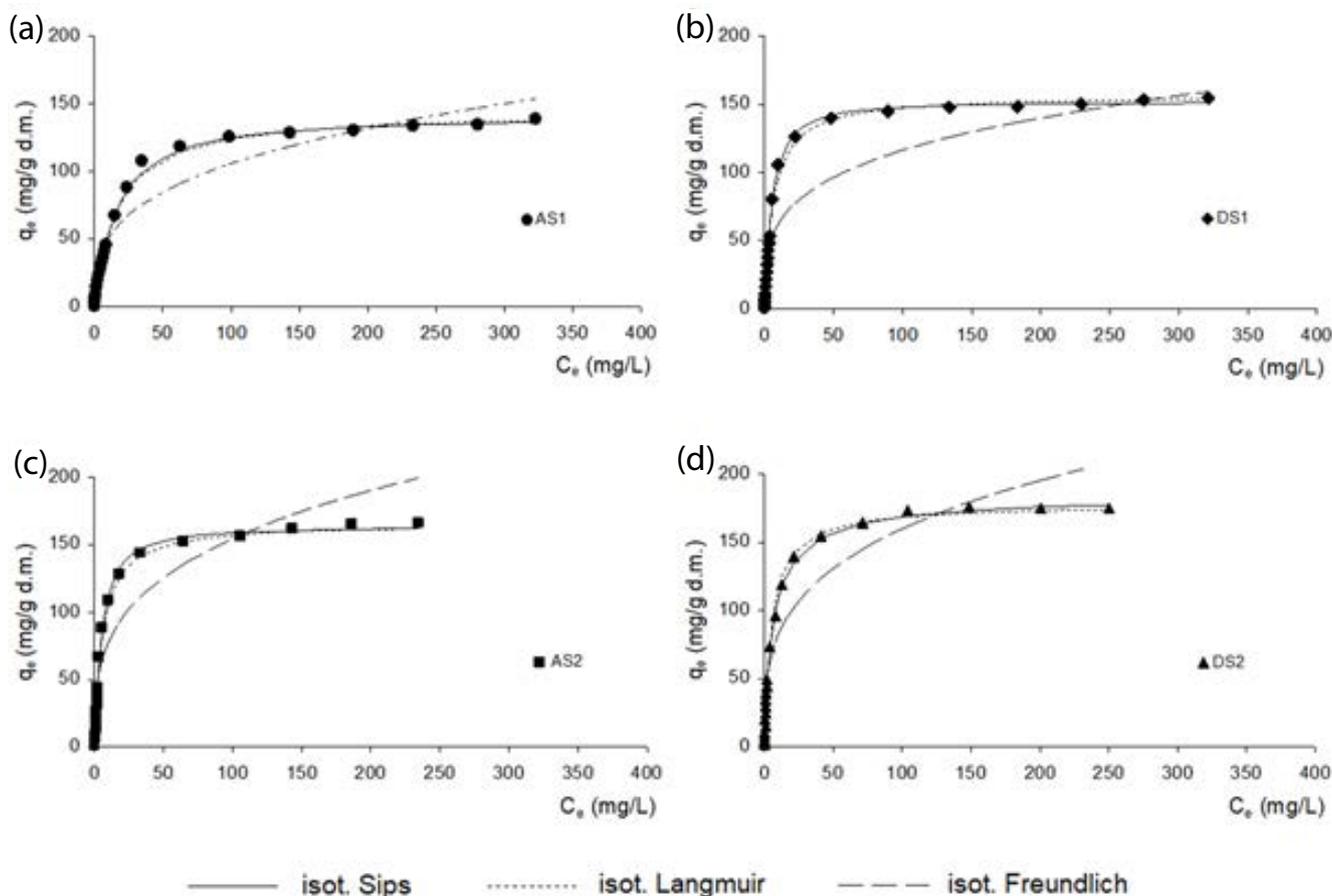
Fig. 7. Experimental data concerning cadmium adsorption and isotherms were determined from Sips, Langmuir, and Freundlich models for the analyzed sludges (pH = 6 ± 0.5, Temp. 25°C).

Activated sludge consists of a population of bacteria and protozoa. An important trait of all microorganisms allowing them to survive in the presence of a toxic concentration of metal is the capability to form biofilms. Extracellular polymeric substances (EPS) present in biofilms as well as cellular membranes and cell walls of the microorganism community possess many sites with cationic and anionic character which induce biosorption by entering into reactions with cadmium ions [54]. The EPS which are secreted by microorganisms during their growth, are composed of such organic substances as polysaccharides, uronic acids, proteins, nucleic acids, and lipids [55]. The anionic character of EPS, resulting from the preponderance of negatively-charged groups, enables the binding of significant amounts of ions of metals, including cadmium. In turn, the cell wall of bacteria is constituted by polymeric substances which contain negatively-charged functional groups like carboxyl, phosphate, and sulfate groups [56]. Multiplicity and diversity of negatively-charged groups in mixed populations of microorganisms of the analyzed sludges resulted in significant values of their maximum adsorption capacity ranging from 141.67 mg/g d.m. (AS1) to 185.58 mg/g d.m. (DS2).

Literature data indicate the highest sorption capacity is exhibited by these microorganisms whose metabolism enables the generation of inorganic ions like sulfides,

carbonates or phosphates. Under such conditions, insoluble salts of metals are being formed that are retained on cell surface, like in the case of cadmium removal by *Alcaligenes denitrificans* [57]. The growth of denitrifying bacteria caused the medium to become alkaline. The extent of cadmium removal from the solution was correlated with pH increase. Authors demonstrated that almost whole cadmium removed from the solution was precipitated in the form of carbonates, which was confirmed by results of X-ray diffraction analysis. High effectiveness of cadmium removal (much more than 1 g/g) was reported by Macaskie and Dean [58,59], who carried out analyses in a column filled with *Citrobacter* sp. cells immobilized in polyacrylamide gel. The adsorption mechanism was associated with the capability of *Citrobacter* sp. for extracellular accumulation of orthophosphates when cultured on the medium containing $C_3H_7O_6PNa_2$. In our study, AS2 and DS2 were cultured on the medium with composition identical to that used in the culture of *Citrobacter* sp. and their sorption capacity of AS2 and DS2 was less and reached 152.3 and 185.6 mg/g d.m., respectively. This may indicate that in the mixed populations of microorganisms the specific substrate – namely 2-phosphate glycerol, was less effective in inducing synthesis of the same enzyme as that synthesized by *Citrobacter* sp. The enzyme (acidic phosphatase) located in the cellular membrane caused the

Table 1
Adsorption constants determined from Langmuir, Freundlich, and Sips equations for the tested sludges

Model	AS1	DS1	AS2	DS2
Langmuir				
q_{max} (mg/g)	144.82	156.77	166.28	177.60
b (L/mg)	0.06	0.16	0.17	0.19
R^2	0.9982	0.9978	0.9978	0.9970
ARE (%)	2.28	3.95	4.31	4.78
Freundlich				
K_F (L/mg)	24.19	33.53	37.86	42.02
n	0.32	0.27	0.30	0.29
R^2	0.9661	0.9474	0.9479	0.9586
ARE (%)	17.1	10.0	13.6	9.20
Sips				
q_{max} (mg/g)	141.67	152.29	164.07	185.58
b (L/mg)	0.05	0.13	0.15	0.20
n	1.08	1.20	1.15	0.85
R^2	0.9984	0.9988	0.9986	0.9981
ARE (%)	1.38	2.49	2.62	3.56

release and retention of HPO_4^{2-} on cell surface and, resultantly, extracellular precipitation of cadmium in the form of insoluble phosphates.

5. Conclusion

Adsorption of cadmium using activated sludge and dried sludge cultured on two types of media was affected by sludge and medium type. The amount of adsorbed metal ions was influenced by solution pH, contact time, and initial concentration of cadmium. Analyses of the toxic effect of cadmium on the enzymatic activity of sludge biomass demonstrated that the greatest decrease in the biochemical activity of activated sludge from culture 2 (AS2). In the sample with the initial cadmium concentration of $C_0 = 150$ mg /L, the TTC value decreased by 82.6% already after 1 min compared to the control sample. The adsorption capacity of the analyzed sludge increased along with pH increase from 2 to 6. The highest adsorption capacity was obtained for dried sludge from culture 2 (DS2) at pH > 6. Cadmium uptake has achieved a very high rate and the adsorption equilibrium was reached after ca. 10 min. Adsorption of Cd(II) on the analyzed biosorbents followed the pseudo-second-order reaction. Inactivation of sludges had a significant effect on the increase in the maximum adsorption capacity, especially in case of sludge from culture 2, that is, from 164.1 mg/g d.m. (AS2) to 185.6 mg/g d.m. (DS2). The higher adsorption capacity of AS2 and DS 2 could be due to the presence of 2-phosphate glycerol in the culture medium which induced the synthesis of acidic phosphatase, which in turn caused the release and retention of HPO_4^{2-} on cell surface and, consequently, extracellular precipitation of cadmium in the form of insoluble phosphates. The significant value of the maximum adsorption capacity, a wide range of

solution pHs, and rate of the adsorption process enable concluding that DS2 sludge may be a promising biosorbent in effective and inexpensive removal of toxic cadmium from wastewater.

References

[1] P.C. Mane, A.B. Bhosle, Bioremoval of some metals by living algae *Spirogyra* sp. and *Spirullina* sp. from aqueous solution, Int. J. Environ. Res., 6 (2012) 571–576.
[2] E. Kordialik-Bogacka, A. Diowksz, Metal uptake capacity of modified Saccharomyces pastorianus biomass from different types of solution, Environ. Sci. Pollut. Res., 21 (2014) 2223–2229.
[3] D. Mohan, K.P. Singh, Single- and multi-component adsorption of cadmium and zinc using activated carbon derived from bagasse – an agricultural waste, Water Res., 36 (2002) 2304–2318.
[4] M. Iqbal, R.G.J. Edyvean, Loofa sponge immobilized fungal biosorbent: a robust system for cadmium and other dissolved metal removal from aqueous solution, Chemosphere, 61 (2005) 510–518.
[5] K. Rao, M. Mohapatra, S. Anand, P. Venkateswarlu, Review on cadmium removal from aqueous solutions, Int. J. Eng. Sci. Technol., 2 (2011) 81–103.
[6] S. Muthusaravanan, N. Sivarajasekar, J.S. Vivek, T. Paramasivan, Mu. Naushad, J. Prakashmaran, V. Gayathri, O.K. Al-Duaij, Phytoremediation of heavy metals: mechanisms, methods and enhancements. Environ. Chem. Lett., 16 (2018) 1339–1359.
[7] A. Saxena, M. Bhardwaj, T. Allen, S. Kumar, R. Sahney, Adsorption of heavy metals from wastewater using agricultural–industrial wastes as biosorbents, Water Sci., 31 (2017) 189–197.
[8] K. Chojnacka, Biosorption and bioaccumulation – the prospects for practical applications, Environ. Int., 36 (2010) 299–307.
[9] E. Kusvuran, D. Yildirim, A. Samil, O. Gulnaz, A study: removal of Cu(II), Cd(II), and Pb(II) ions from real industrial water and contaminated water using activated sludge biomass, Clean – Soil, Air, Water, 40 (2012) 1273–1283.
[10] C.G. Rocha, D.A.M. Zaia, R.V. da S. Alfaya, A.A. da S. Alfaya, Use of rice straw as biosorbent for removal of Cu(II), Zn(II), Cd(II) and Hg(II) ions in industrial effluents, J. Hazard. Mater., 166 (2009) 383–388.
[11] J.C. Zheng, H.M. Feng, M.H.W. Lam, P.K.S. Lam, Y.W. Ding, H.-Q. Yu, Removal of Cu(II) in aqueous media by biosorption using water hyacinth roots as a biosorbent material, J. Hazard. Mater., 171 (2009) 780–785.
[12] D.H.K. Reddy, D.K.V. Ramana, K. Seshaiah, A.V.R. Reddy, Biosorption of Ni(II) from aqueous phase by Moringa oleifera bark, a low cost biosorbent, Desalination, 268 (2011) 150–157.
[13] K. Kishore Kumar, M. Krishna Prasad, G. Rama Lakshmi, C.V.R. Murthy, Studies on biosorption of cadmium on grape pomace using response surface methodology, Desal. Water Treat., 51 (2013) 5592–5598.
[14] N.K. Soliman, A.F. Moustafa, Industrial solid waste for heavy metals adsorption features and challenges; a review, J. Mater. Res. Technol., 9 (2020) 10235–10253.
[15] J. Iqbal, N.S. Shah, M. Sayed, N.K. Niazi, M. Imran, J.A. Khan, Z. Ul Haq Khan, A.G.S. Hussien, K. Polychronopoulou, F. Howari, Nano-zerovalent manganese/biochar composite for the adsorptive and oxidative removal of Congo-red dye from aqueous solutions, J. Hazard. Mater., 403 (2021) 123854, doi: 10.1016/j.jhazmat.2020.123854.
[16] G. Sharma, Mu. Naushad, Adsorptive removal of noxious cadmium ions from aqueous medium using activated carbon/zirconium oxide composite: isotherm and kinetic modelling, J. Mol. Liq., 310 (2020) 113025, doi: 10.1016/j.molliq.2020.113025.

[17] L. Deng, X. Zhu, X. Wang, Y. Su, H. Su, Biosorption of copper(II) from aqueous solutions by green alga *Cladophora fascicularis*, Biodegradation, 18 (2006) 393–402.

[18] A.Y. Dursun, A comparative study on determination of the equilibrium, kinetic and thermodynamic parameters of biosorption of copper(II) and lead(II) ions onto pretreated *Aspergillus niger*, Biochem. Eng. J., 28 (2006) 187–195.

[19] V.J.P. Vilar, C.M.S. Botelho, R.A.R. Boaventura, Modeling equilibrium and kinetics of metal uptake by algal biomass in continuous stirred and packed bed adsorbers, Adsorption, 13 (2007) 587–601.

[20] M.A. Hanif, R. Nadeem, H.N. Bhatti, N.R. Ahmad, T.M. Ansari, Ni(II) biosorption by *Cassia fistula* (Golden Shower) biomass, J. Hazard. Mater., 139 (2007) 345–355.

[21] K.C. Bhainsa, S.F. D'Souza, Removal of copper ions by the filamentous fungus, *Rhizopus oryzae* from aqueous solution, Bioresour. Technol., 99 (2008) 3829–3835.

[22] A. Khosravan, B. Lashkari, Technology, adsorption of Cd(II) by dried activated sludge, Iran. J. Chem. Eng., 8 (2011) 41–56.

[23] S.A. Ong, E. Toorisaka, M. Hirata, T. Hano, Adsorption and toxicity of heavy metals on activated sludge, ScienceAsia, 36 (2010) 204–209.

[24] U. Filipkowska, M. Kuczajowska-Zadrożna, Investigation of the adsorption/desorption equilibria of Cd(II), Zn(II) and Cu(II) ions on/from immobilized digested sludge using biosurfactants, Environ. Earth Sci., 75 (2016), doi: 10.1007/s12665-016-5674-6.

[25] S.A. Ong, C.E. Seng, P.E. Lim, Influence of heavy metal on activated sludge activity: DO and SOUR monitoring, J. Eng. Res. Educ., 4 (2007) 20–24.

[26] E. Liwarska-Bizukojc, Influence of imidazolium ionic liquids on dehydrogenase activity of activated sludge microorganisms, Water Air Soil Pollut., 221 (2011) 327–335.

[28] G.M. Gadd, Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment, J. Chem. Technol. Biotechnol., 84 (2009) 13–28.

[29] P. Yong, L.E. Macaskie, Removal of lanthanum, uranium and thorium from the citrate complexes by immobilized cells of *Citrobacter* sp. in a flow-through reactor: implications for the decontamination of solutions containing plutonium, Biotechnol. Lett., 19 (1997) 251–256.

[30] B. Zhang, S. Xiong, B. Xiao, D. Yu, X. Jia, Mechanism of wet sewage sludge pyrolysis in a tubular furnace, Int. J. Hydrogen Energy, 36 (2011) 355–363.

[31] S.A. Ong, E. Toorisaka, M. Hirata, T. Hano, Effects of nickel(II) addition on the activity of activated slude microorganisms and activated sludge process, J. Hazard. Mater., 113 (2004) 111–121.

[32] K. Matyja, A. Ziembińska, A. Małachowska-Jutsz, Cadmium and copper toxicity assessment in activated sludge using TTC bioassay, Arch. Environ. Prot., 37 (2011) 85–94.

[33] H. Dhaouadi, F. M'Henni, Vat dye sorption onto crude dehydrated sewage sludge, J. Hazard. Mater., 164 (2009) 448–458.

[34] O. Gulnaz, A. Kaya, S. Dincer, The reuse of dried activated sludge for adsorption of reactive dye, J. Hazard. Mater., 134 (2006) 190–196.

[35] W.J. Song, X. Pan, D. Zhang, Lead complexation of soluble and bound extracellular polymeric substances from activated sludge: characterized with fluorescence spectroscopy and FTIR spectroscopy, Biotechnol. Biotechnol. Equip., 26 (2012) 3371–3377.

[36] L. Zhu, M. le Lv, X. Dai, J. heng Zhou, X. yang Xu, The stability of aerobic granular sludge under 4-chloroaniline shock in a sequential air-lift bioreactor (SABR), Bioresour. Technol., 140 (2013) 126–130.

[37] A. Chiş, F. Fetea, A. Taoutaou, C. Socaciu, Application of FTIR spectroscopy for a rapid determination of some hydrolytic enzymes activity on sea buckthorn substrate, Rom. Biotechnol. Lett., 15 (2010) 5738–5744.

[38] X.H. Guan, Q. Liu, G.H. Chen, C. Shang, Surface complexation of condensed phosphate to aluminum hydroxide: an ATR-FTIR spectroscopic investigation, J. Colloid Interface Sci., 289 (2005) 319–327.

[39] D. Zhang, J. Wang, X. Pan, Cadmium sorption by EPSs produced by anaerobic sludge under sulfate-reducing conditions, J. Hazard. Mater., 138 (2006) 589–593.

[40] D. Sonali, K. Jayant, K. Sunil, Activated sludge for heavy metal removal, Int. J. Res. Appl. Sci. Eng. Technol., 2 (2014) 254–259.

[41] X. Zhang, Y. Hao, X. Wang, Z. Chen, C. Li, Competitive adsorption of cadmium(II) and mercury(II) ions from aqueous solutions by activated carbon from xanthoceras sorbifolia bunge hull, J. Chem., 2016 (2016), doi: 10.1155/2016/4326351.

[42] A.V. Ajay Kumar, N.A. Darwish, N. Hilal, Study of various parameters in the biosorption of heavy metals on activated sludge, World Appl. Sci. J., 5 Special Issue Environ., 5 (2009) 32–40.

[43] M.D. Meitei, M.N.V. Prasad, Lead(II) and cadmium(II) biosorption on *Spirodela polyrhiza* (L.) Schleiden biomass, J. Environ. Chem. Eng., 1 (2013) 200–207.

[44] M. Oves, M.S. Khan, A. Zaidi, Biosorption of heavy metals by *Bacillus thuringiensis* strain OSM29 originating from industrial effluent contaminated north Indian soil, Saudi J. Biol. Sci., 20 (2013) 121–129.

[45] J. Iqbal, N.S. Shah, M. Sayed, M. Imran, N. Muhammad, F.M. Howari, M.A. Haija, Synergistic effects of activated carbon and nano-zerovalent copper on the performance of hydroxyapatite-alginate beads for the removal of As³⁺ from aqueous solution, J. Cleaner Prod., 235 (2019) 875–886.

[46] S.A. Ong, E. Toorisaka, M. Hirata, T. Hano, Comparative study on kinetic adsorption of Cu(II), Cd(II) and Ni(II) ions from aqueous solutions using activated sludge and dried sludge, Appl. Water Sci., 3 (2013) 321–325.

[47] H. Zare, H. Heydarzade, M. Rahimnejad, A. Tardast, M. Seyfi, S.M. Peyghambarzadeh, Dried activated sludge as an appropriate biosorbent for removal of copper(II) ions, Arabian J. Chem., 8 (2015) 858–864.

[48] W. Zhou, L. Ren, L. Zhu, Reducement of cadmium adsorption on clay minerals by the presence of dissolved organic matter from animal manure, Environ. Pollut., 223 (2017) 247–254.

[49] K. Vijayaraghavan, Y.S. Yun, Bacterial biosorbents and biosorption, Biotechnol. Adv., 26 (2008) 266–291.

[50] H. Benaïssa, M.A. Elouchdi, Biosorption of copper(II) ions from synthetic aqueous solutions by drying bed activated sludge, J. Hazard. Mater., 194 (2011) 69–78.

[51] S.K. Papageorgiou, F.K. Katsaros, E.P. Kouvelos, J.W. Nolan, H. Le Deit, N.K. Kanellopoulos, Heavy metal sorption by calcium alginate beads from *Laminaria digitata*, J. Hazard. Mater., 137 (2006) 1765–1772.

[52] B. Nagy, C. Mânzatu, A. Măicăneanu, C. Indolean, L. Barbu-Tudoran, C. Majdik, Linear and nonlinear regression analysis for heavy metals removal using *Agaricus bisporus* macrofungus, Arabian J. Chem., 10 (2017) S3569–S3579.

[53] J.S. Chang, R. Law, C.C. Chang, Biosorption of lead, copper and cadmium by biomass of *Pseudomonas aeruginosa* PU21, Water Res., 31 (1997) 1651–1658.

[54] J.J. Harrison, H. Ceri, R.J. Turner, Multimetal resistance and tolerance in microbial biofilms, Nat. Rev. Microbiol., 5 (2007) 928–938.

[55] S. Comte, G. Guibaud, M. Baudu, Biosorption properties of extracellular polymeric substances (EPS) towards Cd, Cu and Pb for different pH values, J. Hazard. Mater., 151 (2008) 185–193.

[56] M. Yunus Pamukoglu, F. Kargi, Removal of copper(II) ions from aqueous medium by biosorption onto powdered waste sludge, Process Biochem., 41 (2006) 1047–1054.

[57] J. Remacle, I. Muguruza, M. Fransolet, Cadmium removal by a strain of *Alcaligenes denitrificans* isolated from a metal-polluted pond, Water Res., 26 (1992) 923–926.

[58] L.E. Macaskie, A.C. Dean, Microbial metabolism, desolubilization, and deposition of heavy metals: metal uptake by immobilized cells and application to the detoxification of liquid wastes, Adv. Biotechnol. Process., 12 (1989) 159–201.

[59] L.J. Michel, L.E. Macaskie, A.C.R. Dean, Cadmium accumulation by immobilized cells of a *Citrobacter* sp. using various phosphate donors, Biotechnol. Bioeng., 28 (1986) 1358–1365.