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Application of biosurfactants for heavy netals leaching from immobilized activated sludge

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Abstract: This study was undertaken to determine the effectiveness of biosurfactants – saponin, tannin and rhamnolipids JBR 515 and 425, for the removal of cadmium, zinc and copper from activated sludge immobilized in 1.5% sodium alginate with 0.5% polyvinyl alcohol. We also established the impact of pH value on biosorbent regeneration with the analyzed biosurfactants and determined the critical micelle concentration (CMC) in solutions containing the biosorbent and biosurfactant and in exact samples with heavy metals. Saponin exhibited the highest effectiveness of metals leaching at pH 1–5, and rhamnosides at pH 5–6. In addition, the study demonstrated a significant effect of the ratio of biosorbent mass to washing agent volume (m/V) on the effectiveness of metals leaching. Of the biosurfactants analyzed, saponin was ca. 100% effective in leaching zinc and copper. The effectiveness of the other biosurfactants was lower and depended on the metal being leached.

Introduction

The necessity of environment protection and economic concerns have made the removal of heavy metals from industrial wastewaters and the recovery of economically-important metals practical issues of key significance.

In recent years, intensive studies have been carried out on the biosorption of metals by biopolymers, biomass and immobilized biomass. Adsorption of copper, cadmium, zinc and lead by biopolymers under static conditions was investigated by Chen et al. (2010), by biomass by Kipigroch et al. (2012) and by immobilized biomass by Kuczajowska-Zadrożna et al. (2004), Tsecova et al. (2010) and Karwowska et al. (2009). Dynamic of heavy metals leaching from soils with sewage sludge addition was also studied (Rosik-Dulewska et al. 2001, Prica et al. 2012).

In practice, immobilized biosorbents should be characterized by high effectiveness of metals adsorption from solutions and by ease of desorption with the use of cheap desorbents, including weak solutions of mineral and organic acids, and complexing agents. Data in the literature indicate that the application of acids leads to the recovery of a good yield of such metals as copper, cadmium and lead (Lezcano et al. 2011). Alternatively many authors have used complexing agents, e.g. EDTA and NTA, for the desorption process (Njikam et al. 2012).

As many desorbing agents cause damage to the structure of biosorbents, a search is underway for new metal-extracting substances. For this reason, increasing attention is being devoted to biosurfactants to leach metals from contaminated soils. Biosurfactants are surface-active compounds of natural origin acquired by microbiological synthesis or extraction from plants.

The biosurfactants produced by bacteria and yeast are biodegradable, and act in a wide range of pH, temperature and environment salinity. They belong mainly to glycolipids, lipoproteins, phospholipids and polymer compounds (Bognolo et al. 1999). As reported by Mulligan and Gibs (1990), a group of biosurfactants synthesized by *Pseudomonas aeruginosa* from substrates containing alkenes (C-11 and C-12), citrate, fructose, olive oil, glucose and mannitol, are rhamnolipids. Their synthesis and composition are determined by the design of a fermentation tank, pH value, substrate and temperature. Commercially-produced rhamnolipids have been applied in the biodegradation of hydrogen carbonates and in the removal of metals from contaminated soils.

The studies were also carried out on elimination of heavy metals from fly ash using leaching solutions containing bacteria producing biological surfactants (Karwowska et al. 2011).

Compared to biosurfactants synthesized by microorganisms, the biosurfactants of plant origin are known to a lesser extent. The most common of the latter include saponin, aescin and tannin.

Saponins are a homogenous group of chemical compounds, representatives of which occur in over 500 plant species from over 90 families. They have been found in both edible (e.g. soybean, spinach) and non-edible plants (e.g. ginseng, chestnut, common soapwort) (Dey et al. 1997). From the chemical perspective, they are glycosides of triterpenoids or sterols, forming the so-called aglycone group (Watanabe et al. 2004). The non-saccharide part of saponins is a system

constituted by 4–6 cyclic carbon rings, in which 1–6 molecules of a monosaccharide usually occur. The most common sugars of saponins are glucose, arabinose, xylose, rhamnose and, less frequently, glucuronic acid (Sahu et al. 2001). The saccharide and non-saccharide parts are linked with an ether bond or, rarely, with an ester bond, and hydroxyl groups of sugars are often acetylated (Dey et al. 1997).

After lignins, tannins constitute the second, most frequent group of polyphenolic compounds occurring in vascular plants (Tharayil et al. 2011). Their concentration depends on species and within a species on age and tissue type (Schweitzer et al. 2008). Their molecular weights range from 500 to 3000 Da, they are generally soluble in water and capable of forming complexes with proteins and polysaccharides, as well as metal ions (Bate-Smith et al. 1962). From the chemical perspective, tannins are a significantly diversified group and their precise division is difficult, nonetheless they are often divided into 2 classes: hydrolyzing and condensed, known as proanthocyanidins (Halvorson et al. 2011, Reed 1995).

The simplest tannins are gallotanins, which contain glucose and gallic acid bound with an ester bond. An example of this type of compounds is tannic acid. The core part of its molecule is constituted by glucose pentagallate, to which successive units of gallic acid are linked by depside bonds (Reed 1995).

In order to apply biosurfactants to the leaching of metals, it is necessary to identify processes to remove metals from biosorbents and to describe the precise conditions for these processes. In view of this, a study was undertaken to investigate the removal of cadmium, zinc and copper form activated sludge immobilized in 1.5% sodium alginate with 0.5% polyvinyl alcohol with the biosurfactants – saponin, tannins and rhamnolipids JBR 515 and 425.

Materials and methods

Preparation of immobilized biomass

The experiment was conducted with excess activated sludges after methane fermentation obtained from a wastewater treatment plant in Olsztyn. The collected sludge was dehydrated by double rinsing with acetone and centrifugation for 10 min at 4500 rpm, and dried in a water bath at a temperature of 50°C for 48 h. Dried sludge was ground in a porcelain mortar and sieved through a screen with a mesh diameter of 0.01 mm. The activated sludge was immobilized to alginate by dissolving 0.5 g of polyvinyl alcohol (PVA) in 80 g of distilled water, and placing in a water bath at a temperature of 60°C. The PVA solution was cooled to a room temperature, 1.5 g of sodium alginate was dissolved, followed by 2 g of the activated sludge and finally distilled water was added to a weight of 100 g. A homogenous sol was instilled to a solution of $0.1 \text{ M}°CaCl_2$ in a saturated solution of boric acid. The grains that formed were left to gelatinize for 24 h, and afterwards rinsed with distilled water in order to wash out chlorides.

Preparation of biosurfactants solution for determination of CMC

Four biological, surface-active compounds were used to study the leaching of heavy metals from immobilized biomass: chemically-pure saponin and tannic acid (*Riedel-deHaën*, Germany) and commercial preparations JBR 515 and 425

(*Jeneil Biosurfactant Co.*, LLC, USA). The JBR 515 and 425 are, respectively, 15% and 25% aqueous solutions of rhamnolipids produced by *Pseudomonas aeruginosa* bacteria during fermentation processes under aerobic conditions.

The critical micelle concentration (CMC) was determined with saponin (50 mg/dm³), tannin (200 mg/dm³), and JBR 515 and 425 (20 mg/dm³).

Determination of the critical micelle concentration (CMC) in aqueous solutions and in exact samples

The critical micelle concentration (CMC) of aqueous solutions of biosurfactants was assayed based on changes in surface tension of these agents before and after the adsorption process. The exact samples contained an aqueous solution of the biosurfactant and grains of the biosorbent. To Erlenmeyer's flask with a volume of 200 cm³, 4 g of the biosorbent were weighed in, and 50 cm³ of a biosurfactant solution were added. Then, the samples were shaken in a Gerhardt type shaker for 2 hours at a constant speed of 170 rpm. Afterwards, the samples were subjected to measurements of surface tension.

The critical micelle concentration (CMC) of the aqueous solutions of biosurfactants and exact samples was determined based on measurements of changes in surface tension, with the plate method of Chu and Chan (2003), using a K100C tensiometer by Krüss (Germany), equipped with LabDesk software.

Effect of concentration, m/V ratio and pH value of desorbing agents on metals removal from biosorbent

A method for the quantitative removal of cadmium, zinc, and copper from biosorbent was conducted as follows. Metal standards at a concentration of 100 mg/dm³ were prepared using distilled water with pH 7.0 adjusted using NaOH and HNO_3. Next, 80 g of the biosorbent was weighed into 1 dm³ of the standard solution, and the sample was mixed on a magnetic stirrer for 12 hours. After adsorption, the biosorbent was rinsed 3 times with distilled water by filtration, dried and mixed with a biosurfactant solution. The samples were placed in a Gerhardt type shaker and shaken for 2 hours at a constant speed of 170 rpm. Afterwards, the samples were collected for assays of heavy metals removal from the biosorbent. The concentration of biosurfactant solutions used to analyze the effect of desorbing agent concentration on metals release from the biosorbent fitted within the following ranges: 1–250 mg/dm³ for saponin, 20–500 mg/dm³ for tannic acid, and 0.25–250 mg/dm³ for JBR 515 and 425.

Analyses of the ratio of biosorbent mass (g) to washing agent volume (V) were conducted at m/V ratios of: 1:5; 1:10; 1:25, and 1:50.

In the case of saponin, the pH value ranged from 1 to 12, whereas in the case of the rhamnolipids JBR 515 and 425 this range was reduced to pH 6–12 owing to their precipitation in the acidic medium.

Determination of heavy metals

Adsorption analyses were conduced with the use of hydrated nitrate salts of cadmium, zinc and copper. Stock solutions of metals were prepared in a concentration of 20 g/dm³ using the following salts: $Cd(NO_3)_2 \cdot 4H_2O$, $Zn(NO_3)_2 \cdot 6H_2O$, and $Cu(NO_3)_2 \cdot 3H_2O$ (Sigma-Aldrich).

The concentration of metals left in the aqueous solution of each of the analyzed samples was controlled with the flame method using an AA 280FS atomic adsorption spectrometer by Varian.

Results and discussion

Determination of the critical micelle concentration (CMC) in solutions of biosurfactant and exact samples

Investigations on the removal of metals from biosorbent were begun with determinations of the critical micelle concentration (CMC) of the analyzed biosurfactant in aqueous solutions of control samples and in exact samples containing the biosorbent (activated sludge immobilized in a mixture of 1.5% alginate and 0.5% PVA) and washing solutions.

Changes in the surface tension depending on log C_{bios} were described with the use of two straight lines. The first line, the so-called rapid changes line, was determined for the range of C_{bios} concentrations at which the biosurfactant occurred in the solution in the form of monomers, whereas the second one, the so-called slow changes line, was determined at C_{bios} concentrations at which micelles were observed to form. The value of log $C_{bios}$ on the axis of abscissae corresponding to the intersection point of the lines, after algorithmization corresponded to the critical micelle concentration (CMC).

The analysis of experimental data demonstrated that initially the surface tension of saponin decreased in proportion to the increasing concentration of saponin (log C_{bios}), until reaching the minimum value (Figure 1).

The minimal value of surface tension occurs when molecules of surface-active compounds fill the whole interface and form a monomolecular layer. Under such conditions, the concentration of surface-active compounds corresponds to the value of the critical micelle concentration (CMC). Once it is exceeded, the surface-active compounds begin to aggregate. The formation of high-molecular aggregates, referred to as micelles, stabilizes the surface tension.

For saponin, the minimal value of surface tension reached 37 mN/m (Figure 2a). In the case of the exact sample, it showed an insignificant ascending tendency along with an increasing saponin concentration, to the value of 37.7 mN/m (Figure 1b).

Tannic acid was characterized by lesser capability to reduce the surface tension which – depending on experimental conditions – was changing in the range from 71.7 to 51.8 mN/m in the control sample (Figure 2a) and from 61.7 to 51.8 mN/m in the exact sample (Figure 2b).
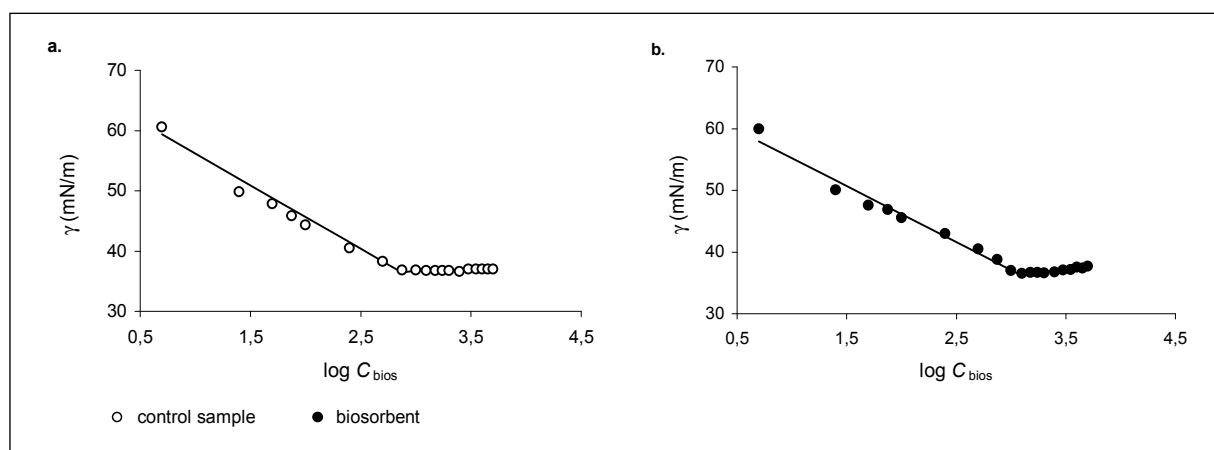
Fig. 1. Changes in surface tension (τ) depending on the concentration of saponin (log C_{bios}) in: a. aqueous solutions (control sample), b. exact samples containing biosorbent
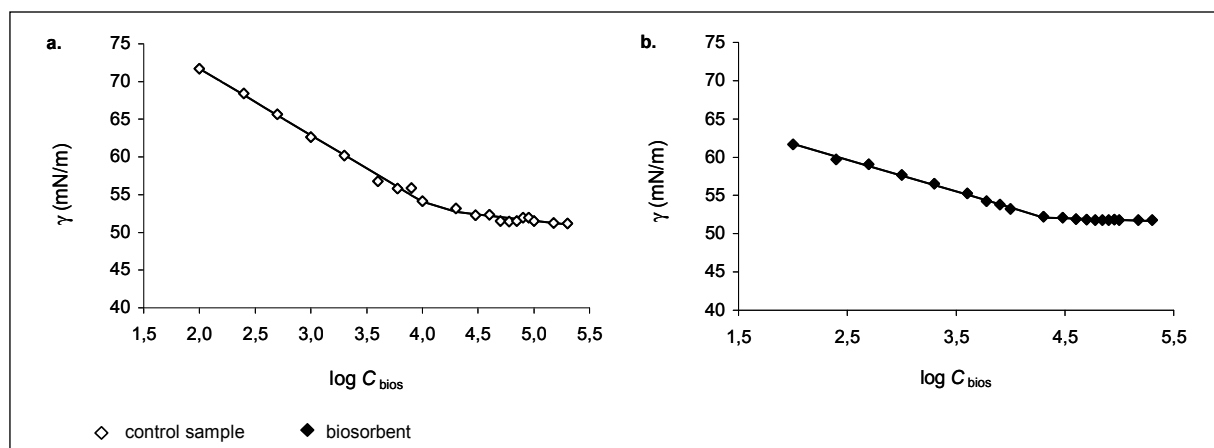
Fig. 2. Changes in surface tension (τ) depending on the concentration of tannic acid (log C_{bios}) in: a. aqueous solutions (control sample), b. exact samples containing biosorbent

Compared to the biosurfactants of plant origin, i.e. saponin and tannic acid, the rhamnolipids demonstrated greater capability to reduce the surface tension. The minimal value of surface tension determined for JBR 515 was 2-fold lower than that assayed for tannic acid (Figure 3).

A similar capability of surface tension reduction was noted in the case of the second analyzed rhamnolipid – JBR 425. The minimal value of surface tension in both the control and exact sample reached 23.0 mN/m (Figure 4).

The study demonstrated that the values of critical micelle concentration (CMC) were affected by the type of analyzed compounds (Table 1). In the control samples containing only biosurfactants, the CMC value was ca. 2-fold lower than in the exact samples. The lowest CMC value, both in the control and exact samples, was determined in the case of rhamnolipids JBR 515 and 425. In contrast, the highest CMC value was assayed for tannic acid. In its case, the CMC value exceeded 200 times (control samples) and 100 times (exact samples) the values noted for JBR 515. In the case of saponin, the achieved values of the critical micelle concentration were intermediate.

Assuming the CMC values obtained for the exact samples as the criterion of establishing the minimal concentration of biosurfactants in washing agents (%), the minimal concentration of saponin in the washing agent was estimated at 0.12%, that of tannin at 2.1% and that of rhamnolipids JBR 515 and 425 − at 0.0234 and 0.0279%, respectively (Table 2).
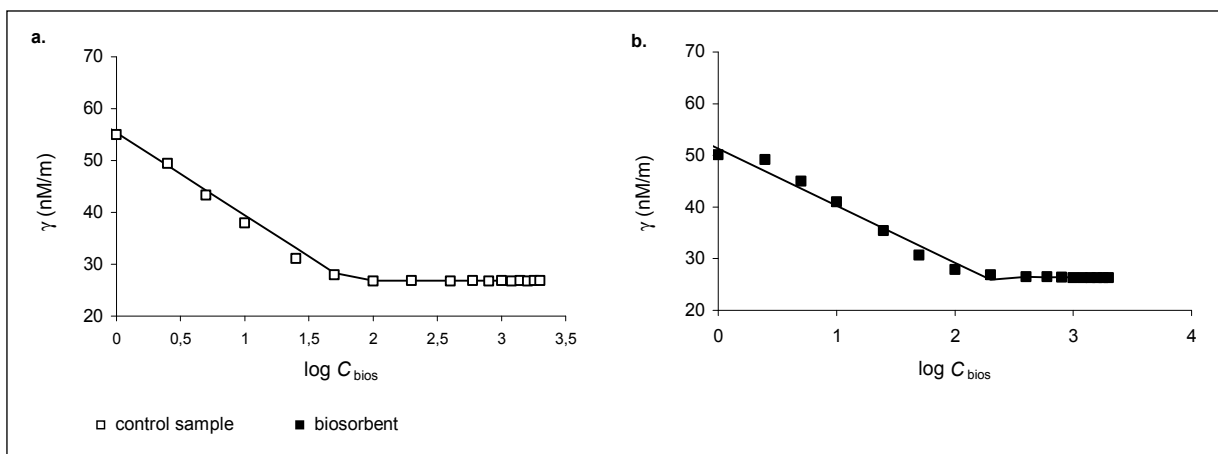

Fig. 3. Changes in surface tension (τ) depending on the concentration of JBR 515 (log C_{bios}) in: a. aqueous solutions (control sample), b. exact samples containing biosorbent]
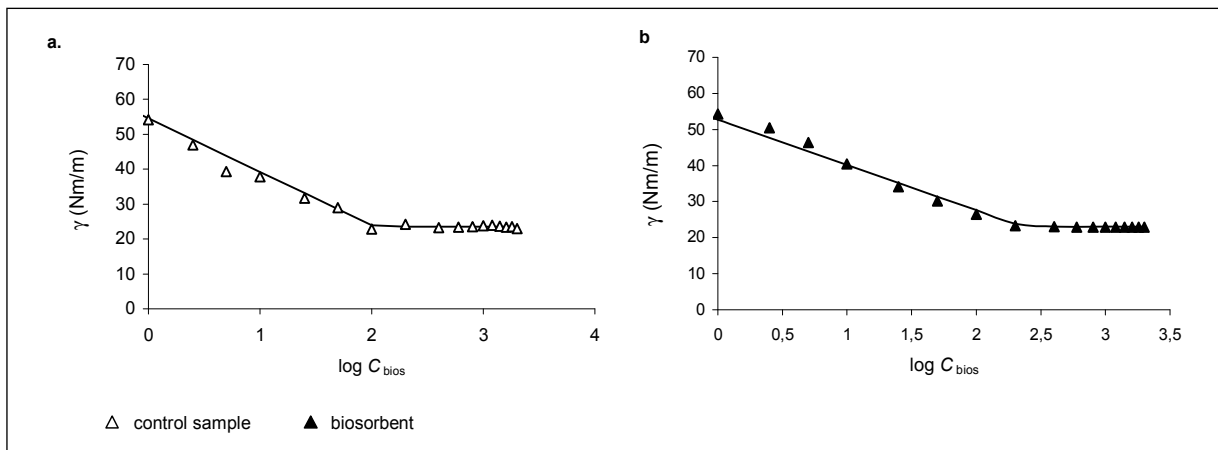

Fig. 4. Changes in surface tension (τ) depending on the concentration of JBR 425 (log C_{bios}) in: a. aqueous solutions (control sample), b. exact samples containing biosorbent]

Table 2. Critical micelle concentration (CMC) of biosurfactants in control and exact samples

Sample	Critical micelle concentration (CMC) (mg/dm^3)			
	saponin	tannic acid	JBR 515	JBR 425
Control	707	13167	63	107
Exact	1206	21633	279	234

Table 1. CMC values of biosurfactants in aqueous solutions acc. to various authors

Type of agent	CMC (mg/dm³)	Reference
15% solution of rhamnolipids	20	(Urum et al. 2004)
Tannin	8	
Saponin from *Quillaja bark*	1000	
Rhamnolipid R1 with molecular weight of 504	50.4	(Helvaci et al. 2004)
Rhamnolipid R2 with molecular weight of 650	97.5	
Rhamnolipid R1 with molecular weight of 504 ATCC9027	50.4	(Ochoa-Loza et al. 2007)
Mixture of R1 and R2 with molecular weight of 588 UG	58.8	
JBR 515	21.9	(Nguen et al. 2008)
JBR 425	230.8	(Ozdemir et al. 2004)
JBR 425	50	(Asci et al. 2007)

The literature data indicate that the commercially-available surface-active biological compounds differ in terms of production method and degree of purity. According to Patist et al. (2000), the presence of impurities in commercial products affects a reduction in the critical micelle concentration owing to rapid saturation of liquid surface at the water-air interface. In the reported study, use was made of chemically-pure saponin and tannic acid as well as of technical products, namely JBR 425 (25%) and JBR 515 (15%), which may explain differences in the CMC values achieved compared to findings of other authors (Table 2).

So far, the structure of 6 types of rhamnolipids synthesized by *Pseudomonas aeruginosa* has been identified and documented. Particular types differ in the number or chemical structure of hydrocarbon chains attached to rhamnose. The R3 and R4 types contain one hydrocarbon chain, whereas types R1 and R2 – two chains, and finally RA and RB types – three chains (Lang et al. 1999). The JBR 425 and 515 rhamnolipids applied in this study constituted a mixture of R1 and R2 types.

Saponins may originate from plant sources including bark of Indian soapberry, grass and tea grains (Hong et al. 2000). The type of raw material affects the chemical composition of products and, consequently, their CMC value. When comparing results of our study with those of other authors, it ought to be concluded that the value of the critical micelle concentration of saponin did not differ significantly from literature data.

Tannins are constituents of plant biomass. According to Ramakirshana et al. (2006), almost all plants contain some tannins, however only few of them in sufficient quantities to enable their application on the commercial scale.

Depending on the type of raw material, tannins differ in their chemical reactivity and biological activity, hence differences are expected as well in their CMC values. In the present study, the value of critical micelle concentration of tannic acid differed significantly from that reported by Urum and Pekdemir (2004).

Most authors claim that the main reason behind CMC increase is the sorption of surface-active compounds on mineral and organic constituents of soil (Schippers et al. 2000). Sorption results in the removal of biosurfactants from the solution. Correspondingly to sorption effectiveness, the concentration of this solution should be respectively higher to enable the process of micellization.

The increase in the CMC value in the exact samples was, therefore, probably due to sorption of biosurfactants in grains of activated sludges immobilized in a mixture of alginate with polyvinyl alcohol.

The best sorbed biosurfactant turned out to be JBR 515, for which sorption effectiveness reached 77.4%. In turn, the sorption effectiveness of saponin was almost 2-fold lower (41.3%), likewise that of tannic acid (39.1%), whereas sorption effectiveness of JBR 425 accounted for 54.3%.

Research by Rodríguez-Cruz et al. (2005) demonstrated that synthetic, non-ionic, surface-active compounds were more strongly adsorbed on soils compared to the anionic compounds. This has, however, not been confirmed in literature data for biosurfactants. Urum and Pekdemir (2004) investigated sorption of aescin, lecithin, rhamnoside, saponin and tannin in sandy soil polluted with petroleum. They showed that in the case of non-ionic saponin from *Quillaja bark* with a purity degree of 10%, the sorption accounted for 66.7%, whereas in the case of anionic rhamnolipids with a concentration of 15% – it was higher and reached 75%. Analogous tendencies were observed in our study, i.e. higher sorption of rhamnolipids compared to saponin and tannic acid.

Differences in sorption efficiency in the present study, when compared to the data of the authors cited above, result from different experimental assumptions including, e.g. concentrations of biosurfactants, mass of biosorbent in the samples, and purity of compounds applied.

Effect of desorbents concentration on the effectiveness of metals removal from biosorbent

The adopted criterion characterizing the effectiveness of test biosurfactants was the efficiency of metals washing out from biosorbent. In the reported study, the removal of metals from immobilized activated sludge using the test biosurfactants in concentrations corresponding to CMC values turned out to be little effective. The increase in concentrations of saponin and both rhamnolipids in the aqueous solution to 5% caused an increase in the effectiveness of removal of all analyzed metals. The successive increase of concentration had no longer such a great impact on process effectiveness. Similar effects were achieved for tannic acid, in which case effectiveness increased significantly until the concentration of 50% (Figure 5).
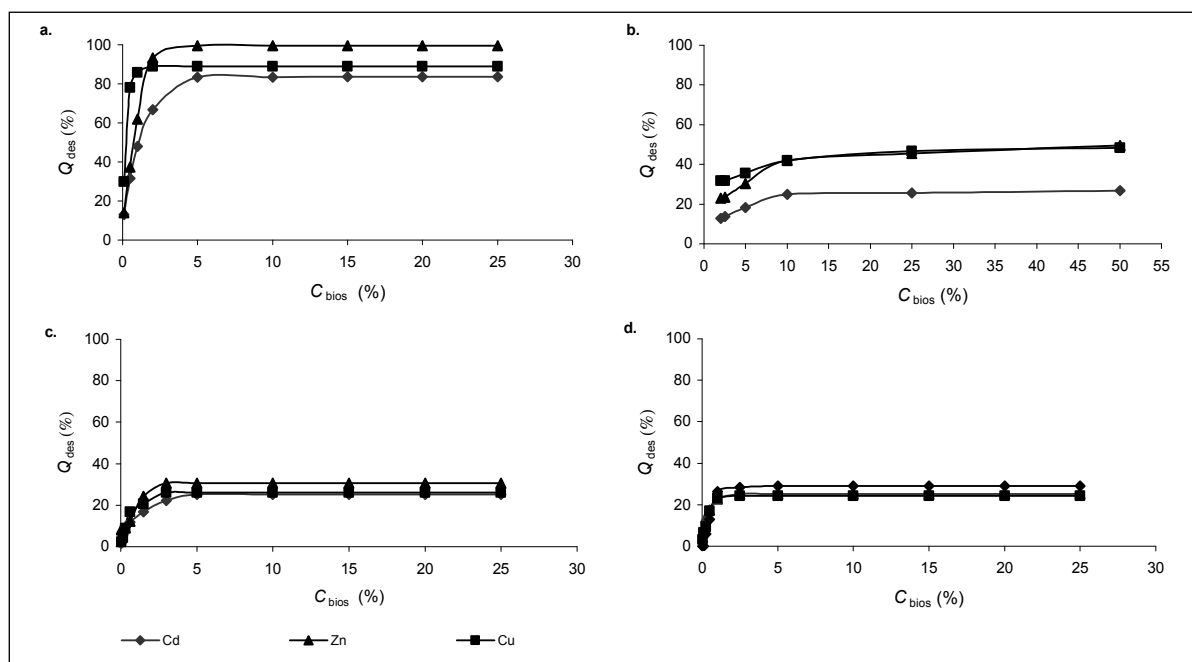
Fig. 5. Effectiveness of metals removal (Q_{des}) from activated sludge immobilized in a mixture of 1.5% alginate with 0.5% PVA depending on biosurfactant concentration (C_{bios}) using solutions of: a. saponin, b. tannic acid, c. JBR 515, and d. JBR425]

The most effective desorbent out of all analyzed biosurfactants turned out to be saponin, for which the effectiveness of desorption of cadmium, zinc and copper in the concentration of 5% accounted for 83.5, 99.5, and 89.0%, respectively. A significantly lower effectiveness of zinc and copper leaching was reported for tannic acid, in the case of which desorption effectiveness of both these metals did not exceed 50%. In turn, only 26% of cadmium was removed from the immobilized activated sludge. Rhamnolipids JBR 515 and 425 were effective in removing ca. 30% zinc and 25% cadmium and copper from the biosorbent.

The experimental data achieved indicate that in the range of concentrations the analyzed saponin was characterized by a higher capability for metals removal from the immobilized activated sludge, compared to the tested biosurfactants. The investigated range of concentrations enabled us to determine optimal concentrations of desorbing agents, that is above which the effectiveness of the process did not increase.

Based upon our results, the decreasing effectiveness of leaching a series of metals from the activated sludge with the described washing agents is as follows:

saponin, tannic acid, JBR 515 – Zn > Cu > Cd

JBR 425 – Cd > Zn > Cu

The literature data indicate that along with an increasing concentration of surface-active compounds in a washing solution above the CMC, the solubility of organic substances increases proportionally to their concentration, which is linked with their solubilization in micelles (Bajpai et al. 2007). In the case of metals, their removal from soil may be due to ionic exchange, precipitation-solubilization or counter-ions exchange (Doong et al. 1998).

In this study, a model is postulated that is based on the analysis of equilibrium of adsorption and desorption of anionic biosurfactants adsorbing on the sludge immobilized on alginate with PVA, onto which cadmium, zinc and copper were adsorbed previously. This is directly linked to the changes in concentrations of metals released from the sorbent applied. Table 3 presents a 3-stage mechanism elucidating the leaching of heavy metals ions adsorbed on the surface of a sorbent using alkaline biosurfactants, doses of which exceeded the CMC value in an aqueous solution.

At the first stage, molecules of the biosurfactant from dissociated micelles may easily adsorb on the interfacial surface. After some time, they are subject to desorption and re-form micelles in the aqueous solution. By this means, a dynamic equilibrium is being established. This process enables acid-base and electrostatic interactions of charges between hydrophilic anions of biosurfactants and cations of metals bound at the active sites of the sorbent. Additionally, non-ionic hydrophilic polar groups of the biosurfactant molecules and sorbent surface interactions occur, at which no sorption of metals occurred, until the moment of the formation of a biosurfactant monolayer covering the sorbent. The second stage involves competitive adsorption of biosurfactant molecules and metal ions on the sorbent. The adsorption of biosurfactant molecule proceeds at head-head and tail-tail positions. It is claimed that interruption of interactions between metal ions adsorbed on the sorbent results from a high gradient of voltage induced by a biosurfactant film on the sorbent's surface (Wasan et al. 2003). The last stage begins with the spatial translocation of a biosurfactant film at the sorbent-water interface, thus causing the leaching of metal ions and their unassisted binding into metal-biosurfactant complexes as a result of chemi-micelle aggregation on the top of the monolayer and, finally, desorption of metal-biosurfactant complexes. The development of chemi-micelles into complete micelles proceeds spontaneously due to increased electrostatic interactions with neighboring molecules of chemimicelles and/or cation exchange inside the double layer. Finally, there occurs desorption of micelles formed and/or metal-

Table 3. Mechanism of metals leaching from biosorbent with the use of alkaline biosurfactants

Biosurfactant solution in the concentration exceeding CMC with absorbed metal	3-stage mechanism of metals leaching from biosorbent		
	Stage 1	Stage 2	Stage 3
	Adsorption of biosurfactant molecules from dissociated micelles on biosorbent	Adsorption of biosurfactant at head-head and tail-tail position	Aggregation of chemimicelles and desorption of metal--biosurfactant complexes

Table 4. Effectiveness of metals removal from activated sludge immobilized in a mixture of 1.5% alginate with 0.5% PVA (Q_{des}) at varying ratio of biosorbent mass to washing agent volume (m/V)

Biosorbent mass to washing agent volume m/V	Mass of metal desorbed from biosorbent (%)		
Saponin	**Cd**	**Zn**	**Cu**
1:5	25.8	47.2	46.5
1:10	37.4	73.6	58.5
1:25	53.2	86.0	81.0
1:50	91.0	96.0	95.0
Tanin			
1:5	3.9	10.1	21.0
1:10	5,8	14,6	19.8
1:25	9.7	27.7	23.3
1:50	20.9	35.4	25.3
JBR 515			
1:5	20.6	24.0	14.0
1:10	21.3	22.4	14.0
1:25	23.0	35.0	20.0
1:50	24.9	42.0	26.0
JBR 425			
1:5	13.1	16.0	11.8
1:10	21.0	24.5	12.1
1:25	21.8	30.0	15.2
1:50	**25.1**	**39.1**	**15.0**

biosurfactant aggregates from the sorbent, with constant transfer of metal ions to the solution as a result of electrostatic repulsion and surface diffusion by detachment from the negatively-charged biosurfactant film occurring on the surface of the sorbent (Chen et al. 2008).

Effect of the ratio of biosorbent mass to washing agent volume (m/V) on the effectiveness of metals removal from biosorbent

The effect of the varying ratio of biosorbent mass to washing agent volume (m/V) was evaluated based on the percentage concentration of metal released from the biosorbent ($Q_{des.}$) (Table 4).

The experimental data demonstrate that in the case of saponin, varying the m/V ratio exerted the strongest impact on cadmium desorption. Increasing the m/V ratio from 1:5 to 1:50, increased the quantity of removed cadmium from 25.8% to 91.0%. The effect of changes in sample dilution with a saponin solution on Q_{des} values was less with zinc and copper. The efficiency of heavy metals desorption with the use of tannic acid was significantly lower than in the case of saponin. The change in dilution rate of the samples was observed to significantly affect the effectiveness of cadmium and zinc removal from 3.9% (1:5) to 20.9% (1:50) and from 10.1% (1:5) to 35.0% (1:50), respectively. In turn, in the case of copper, the increase in the dilution rate of samples did not elicit any significant changes in process effectiveness. The analysis of metals desorption with the use of rhamnolipids JBR 515 and 425 demonstrates that in the case of cadmium and copper, the effect of changes in the m/V ratio on Q_{des} values was not as significant as in the case of zinc. Taking into account the most effective ratio of biosorbent mass with absorbed metals

to washing agent volume (1:50), the capability of the analyzed biosurfactants for cadmium, zinc and copper desorption was ordered as follows:

Cd: saponin > JBR 425 >JBR 515 > tannic acid
**Zn:** saponin > JBR 515 > JBR 425 > tannic acid
Cu: saponin > JBR 515 > tannic acid > JBR 425

Sample dilution causes a decrease in the volume of washing agents that may be subject to sorption, as well as increasing the contact between the contaminated medium and the washing solution, which has a positive impact on the effectiveness of metals removal.

The study demonstrated that, irrespective of the type of biosurfactants and metal, the increasing volume of the washing agent improved the effectiveness of metal removal from the immobilized activated sludges.

Effect of pH value on biosorbent regeneration with the use of biosurfactants

Experimental data showing the effectiveness of metal leaching with the use of the analyzed biosurfactants in control samples (water with specified pH) and in exact samples depending on pH value are presented in Figure 6.
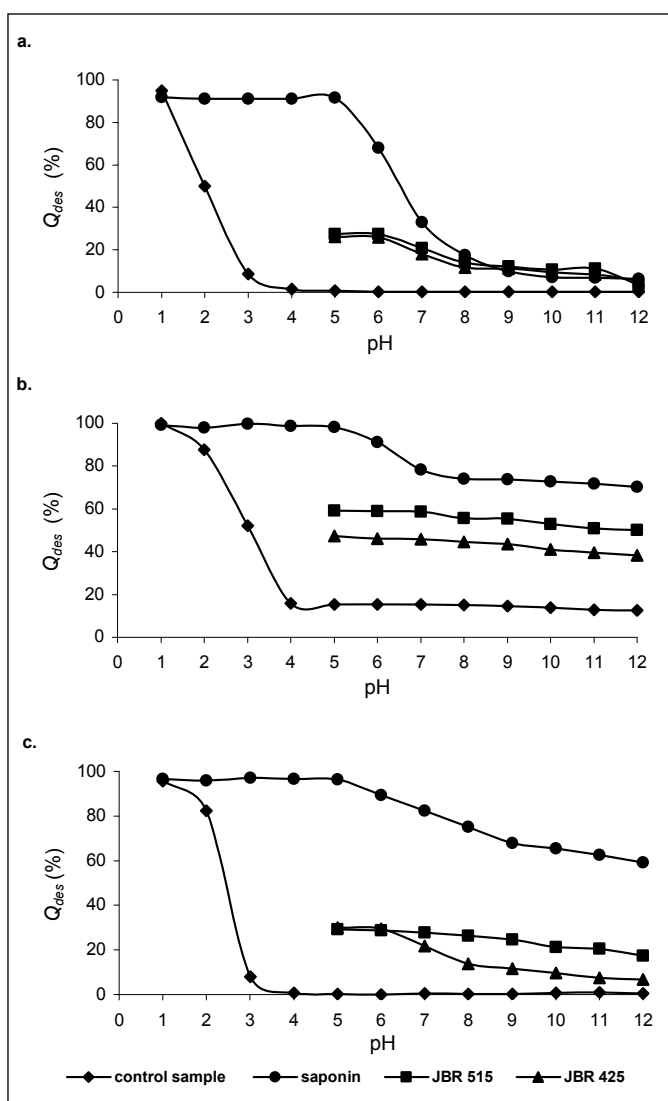
Fig. 6. Effectiveness of metals leaching: a. cadmium, b. zinc, c. copper, from biosorbent with the use of biosurfactant solutions at different pH values]

The study demonstrated that in the control samples, at pH values between 4–12, the effectiveness of metals removal from the biosorbent was low. In the case of zinc it did not exceed 15.5% (Figure 6b), whereas in the case of cadmium and copper it reached 1.1% on average. After pH value drop to 1, a significant increase was noted in the effectiveness of metals removal: Cd – 95%, Zn – 100%, and Cu – 95.6%.

Compared to the control sample, a similar effectiveness of the process was achieved for saponin but in a wider pH range, i.e. pH 1 – pH 5, especially at pH 5, which points to a beneficial role of saponin in metals leaching at this pH.

An increase of pH value to pH 12 resulted in a significant drop in effectiveness, especially in the case of cadmium removal (6.4%). In the case of zinc and copper, the effectiveness also decreased, respectively, 70% and 59% (Figure 6 b, c). For this reason, pH 5 was found to be optimal. Moreover, the solution of saponin had the same pH value (pH 5) and therefore the samples did not require additional pH adjustment. In addition, at this pH value, the greatest differences were noted in the effectiveness of metals leaching between the control sample and exact samples, which means that the presence of saponin in the solution was the factor determining process effectiveness.

An overview of the literature data indicates that saponin desorbed heavy metals more effectively at lower pH values. However, the pH values may differ depending on the type of medium being washed and on technological conditions. For instance, Hong et al. (2002) analyzed the desorption of copper, zinc and cadmium from contaminated soils using saponin and achieved the highest effectiveness of copper and zinc removal at pH 3, whilst 100% desorption of cadmium – at pH 3.9. It was additionally noticed that at pHs above 6, the process practically did not proceed. In turn, Chen et al. (2008) reported that the highest effectiveness of copper desorption from kaolin by saponin was achieved at a pH range of 2 to 8. In their study, the maximum desorption of Cu (over 90%) was obtained at pH 2, but pH 6.5 was resulted in 83% desorption of Cu).

The impact of pH value on the effectiveness of metals removal with the use of rhamnolipids JBR 515 and JBR 425 was complex and depended on the type of metal (Figure 8). In the case of all analyzed metals, both biosurfactants tested exhibited the highest removal effectiveness at pH 5–6 (Cd 267%, Zn − 55–743%, and Cu − 29.5%). Increasing the pH to 12 had a negative effect of the effectiveness of metals removal.

Taking into account the effectiveness of metals washing, pH 6 was adopted as optimal for both rhamnolipids examined. At pH 6, analogously to pH 5.0 for saponin, the greatest differences were recorded in the effectiveness of metals leaching between the control sample and exact samples, which means that the presence of JBR 515 and 425 in the solution was the factor determining process effectiveness.

A similar value of the most favorable pH, i.e. pH 6.8, for the leaching of cadmium from kaolin with the use of JBR 425 was achieved by Asci et al. (2007). In turn, Mulligan and Wang (2004) report that at pH 6.8 the analyzed rhamnolipid was capable of removing 73.2% of Cd and 68.1% of Ni, and that the change of pH from 6.8 to 10 did not result in process effectiveness improvement.

Conclusions

The critical micelle concentration (CMC) of biosurfactants in the aqueous solution was observed to depend on their chemical structure and ionic character. The biosurfactants exhibited sorption capability on the activated sludge immobilized in 1.0% alginate with 0.5% polyvinyl alcohol (PVA), conditions that increased critical micelle concentration. The highest adsorption was noted for JBR 515, for which the CMC value increased 4.4-times. The change in the m/V ratio from 1/5 to 1/50 affected an increase in metals leaching effectiveness. This was due to the increased surface of contact between the biosorbent and the washing agent.

Acknowledgments

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Zastosowanie biosurfaktantów do wymywania metali ciężkich z immobilizowanego osadu czynnego

W pracy podjęto badania nad określeniem efektywności wykorzystania biosurfaktantów – saponiny, taniny oraz ramnolipidów JBR 515 i JBR 425 do wymywania kadmu, cynku i miedzi z osadu czynnego immobilizowanego w alginianie sodu 1,5% z alkoholem poliwinylowym 0,5%. Przeprowadzone badania pozwoliły określić wpływ odczynu na regenerację biosorbentu za pomocą testowanych biosurfaktantów, wyznaczyć krytyczne stężenie micelarne (CMC) w roztworach zawierających biosorbent i biosurfaktant oraz w próbach właściwych z metalami ciężkimi. Saponina charakteryzowała się najwyższą efektywnością wymywania metali przy pH 1–5, a ramnolipidy przy pH 5–6. Ponadto badania wykazały istotny wpływ stosunku masy biosorbentu do objętości eluatu (m/V) na skuteczność wymywania metali. Spośród analizowanych biosurfaktantów saponina wykazywała około 100% efektywność usuwania z biosorbentu cynku i miedzi. Skuteczność innych biosurfaktantów była niższa i zależała od rodzaju metalu.