Bioleaching of Selected Metals from Waste Printed Circuit Boards by Fungs

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Abstract

The growing demand for non-ferrous metals over the last centuries has resulted in constant extraction of natural resources - in case of many crucial and most widely used raw materials, accessible and high-quality deposits are already close to being depleted. Waste electrical and electronic equipment (WEEE) constitutes a rich, secondary source of metals, the amount of which in the EU is increasing every year. In order to increase resource efficiency and contribute to a circular economy, it is necessary to improve the processing and recycling of waste electrical and electronic equipment at the end of an electronic lifetime. The choice of a suitable method of processing this waste is vital due to the complex and materially diverse composition of WEEE. Waste printed circuit boards (WPCBs) that constitute approx. 3-5% of WEEE by weight are of particular importance from both environmental and economic point of view.

The article investigates the recovery of Cu, Ag and Al from WPCBs using an industrial fungal strain of Aspergillus niger. The bioleaching process was carried out using 3 methods (one-step, two-step and spent medium) in an incubator with shake depending on the contact time and pulp density.

The research presented in the article aimed at assessing the usefulness of the biotechnological method for leaching of selected metals from e-waste. The results indicate that it is possible to mobilise metals from the WPCBs using microorganisms.

Keywords: *metals bioleaching, waste printed board, fungi*

Introduction

Metals are important in all aspects of our daily life. According to the OECD's global forecasts concerning material requirements by 2060, the global demand for non-ferrous metals will increase faster than demand for any other raw material – from 7 to 19 gigatonnes per year by 2060. This is due to social change, growing world population and, above all, new technologies – non-ferrous metals are vital for the transition to a low-carbon economy due to their use in breakthrough technologies such as electric vehicles, renewable energy sources and batteries. The growing demand for non-ferrous metals over the last centuries has resulted in constant extraction of natural resources - in case of many crucial and most widely used raw materials, accessible and high-quality deposits are already close to being depleted. What is more, the extraction and processing of non-renewable raw materials involves interference in the environment (Pollmann et al., 2018)

A relevant approach concerning saving resources seems to be recycling. It allows for reusing raw material that has already been exhausted. Waste produced becomes a renewable, secondary source of natural resources which limits its depletion. Moreover, effective recycling provides safe resources for industrialised countries and reduces dependence on resource-rich countries (Dodson et al., 2012; Woynarowska, Żukowski, 2012).

Waste electrical and electronic equipment (WEEE) constitutes a rich, secondary source of metals, the amount of which in the EU is increasing every year from about 9 million tonnes produced in 2005 to the expected more than 12 million tonnes in 2020. (https://ec.europa.eu/environment/waste/weee/index_en.htm). In order to increase resource efficiency and contribute to a circular economy, it is necessary to improve the processing and recycling of waste electrical and electronic equipment at the end of an electronic lifetime. The choice of a suitable method of processing this waste is vital due to the complex and materially diverse composition of WEEE. Waste printed circuit boards (WPCBs) that constitute approx. 3–5% of WEEE by weight are of particular importance from both environmental and economic point of view. They contain, on average, 30-40% of metals by weight, with higher purity than in minerals, including base metals (Cu, Zn), precious metals (Au, Ag, Pd) and heavy metals.

To recycle waste printed circuit boards (WPCBs), mechanical, chemical, and biological methods can be used. Traditional pyrometallurgical and physical separation methods are energy-intensive, while hydrometallurgical methods generate large amounts of chemical waste. (Zhang Y. et al., 2012; Hao J. et al., 2020; Kaya M., 2017; Li H. et al., 2018; Lu Y., Xu Z., 2016; Akcil A. et al., 2015, Cui J, Zhang L, 2018). Therefore, it seems that biohydrometallurgical methods which can be described as ecological, inexpensive, "low-tech" processes with low emission of hazardous substances, using naturally occurring microorganisms and their metabolic products to extract metals from the matrix, have a chance to be applied on an industrial scale. These methods have already been successful in the processing of low-quality ores and bioleaching of industrial solid waste (Xi M. et al., 2018; Pollmann et al., 2018).

The microorganisms involved in the process of bioleaching of WPCBs are mainly chemoautothrops Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans (Brandl H. et al., 2001; Hong Y., Valix M., 2014; Yang Y. et al., 2014), iron-oxidizing bacteria and sulfur bacteria (Wang S. et al., 2016; Kar-

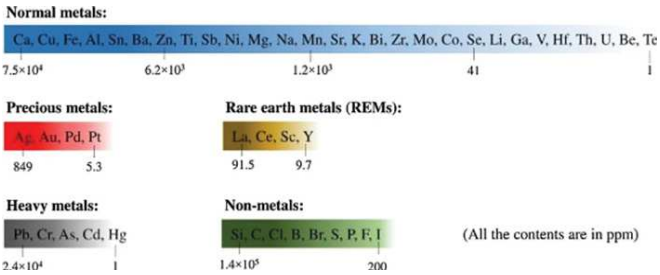

Fig. 1. Contents of metallic and non-metallic elements in typical WPCBs (metal contents decrease with the colour becoming light) (Li H. et al., 2018)
Rys. 1. Zawartość składników metalicznych i niemetalicznych w typowych WPCB (zawartość metali zmniejsza się wraz z rozjaśnianiem się koloru) (Li H. i inni, 2018)

wowska E. et al., 2014; Xia M.C. et al., 2017; Willscher S. et al., 2007; Shah M.B. et al., 2015; Ilyas S. et al., 2010; Xiang Y. et al., 2010; Zhu N. et al., 2011) heterotrophic bacteria Chromobacterium violaceum (Faramarzi M.A., et al., 2004; Chi T.D. et al., 2011) and mould fungus Aspergillus niger (Jadhav U. et al., 2016; Brandl H. et al., 2001; Faraji F. et al., 2017; Kolenčík M. et al., 2013), Penicillium sp. (Ilyas S., Lee J.C., 2013; Brandl H. et al., 2001) and Rhizopus sp. (Netpae T., Suckley S. et al., 2019)

The article investigates the recovery of Cu, Ag and Al from WPCBs using an industrial fungal strain of Aspergillus niger. The bioleaching process was carried out using 3 methods (one-step, two-step and spent medium) in an incubator with shake depending on the contact time and pulp density.

Materials and methods

Pretreatment of WPCBs

Printed circuit boards were separated from mobile phones manually and then crushed in a hammer mill. Three-stage shredding was performed. The first stage involved shredding using a 15mm sieve. The shredded material was classified on a 1mm sieve. Sieving resulted in obtaining a final product with a grain size of 0–1mm and retained with a grain size >1mm, which was put back to the mill. Before the second stage, the sieve was changed to a 5 mm one. The product was sieved, and the results were the same as in the previous stage – grain size 0-1mm. Grains larger than 1mm were put back to the mill. In the last stage, a 1mm sieve was used. Grain size was classified as 0–1mm. Shredding process using hammer mill made it possible to separate the grain size classes, which were subjected to further investigation. The process diagram is shown in Figure 2. During shredding, dust collectors collected dust, which, due to its low weight, constituted a ready product for examination using microorganisms. Shredded material with grain size >1mm was directed to a magnetic separator, where a magnetic and non-magnetic material were detached, which, when combined with the dust collected during the shredding process, was a source for further biological research. Next, in order to ensure sterility, the feed was cleansed using deionised water and placed in the dryer at a temperature of 80oC for 24h.

Chemical analysis

The WPCBs sample was dissolved using aqua regia and then the obtained solution was filtered and analysed for metal content using the Philips PU-9100x atomic absorption spectrophotometer. Obtained results are presented in Table 1.

To analyse the metal content of the bioleaching process, 5 ml of leaching solution was centrifuged at 10,000 RPM for 15 minutes in order to separate the mycelium and then filtered using syringe filters to completely remove solids. The supernatant obtained in such a way was analysed for the average metal content using Philips PU-9100x atomic absorption spectrophotometer.

The medium's pH change was monitored using WTW InoLab® Multi 9310.

Metal recovery at each step was calculated according to the following equation:

$$Bioleaching\ efficiency\ (\%) = C_1/C_0 \cdot 100$$

where C_0 and C_1 are concentrations of metals in the solution before and after bioleaching, respectively.

Microorganisms and growth condition

The A. niger 419 strain used in the research was obtained from the Institute of Agriculture and Food Biotechnology's microbe culture collection. The strain was cultured on potato dextrose agar (PDA) BTL P-0134 at 28°C for 7 days. After the set incubation time, spores were collected by cleansing the surface with deionised water. The obtained suspension was diluted to 107 spores/ml (inoculum). The number of spores was counted using the microscope (magnification 400x) applying a haemocytometer.

Shake flask one-step bioleaching

Inoculum with a concentration of 1ml/100ml of medium was added to 500ml Erlenmeyer flasks containing 200ml of Sabouraud broth BTL P-0133 and various pulp densities of WPCBs 0.1; 0.5; 1; 2.5; 5% w/v, and incubated for 25 days. During a given period of the process, samples of the leaching solution were taken at selected intervals in order to analyse the metal content.

Shake flask two-step bioleaching

In two-step bioleaching, pre-cultivation was carried out by adding an inoculum at a concentration of 1ml/100ml of medium to a 500ml Erlenmayer flask containing 200ml of Sabouraud broth BTL-P-0133 with no WPCBs. After 5 days, the WPCBs with the concentration of 2.5% w/v was added and the obtained suspension was incubated for 20 days. During a given period of the process, samples of the leaching solution were taken at selected intervals in order to analyse the metal content.

Shake flask spent medium bioleaching

Pre-cultivation involving adding an inoculum at a concentration of 1ml/100ml of feed to a 500ml Erlenmayer flask

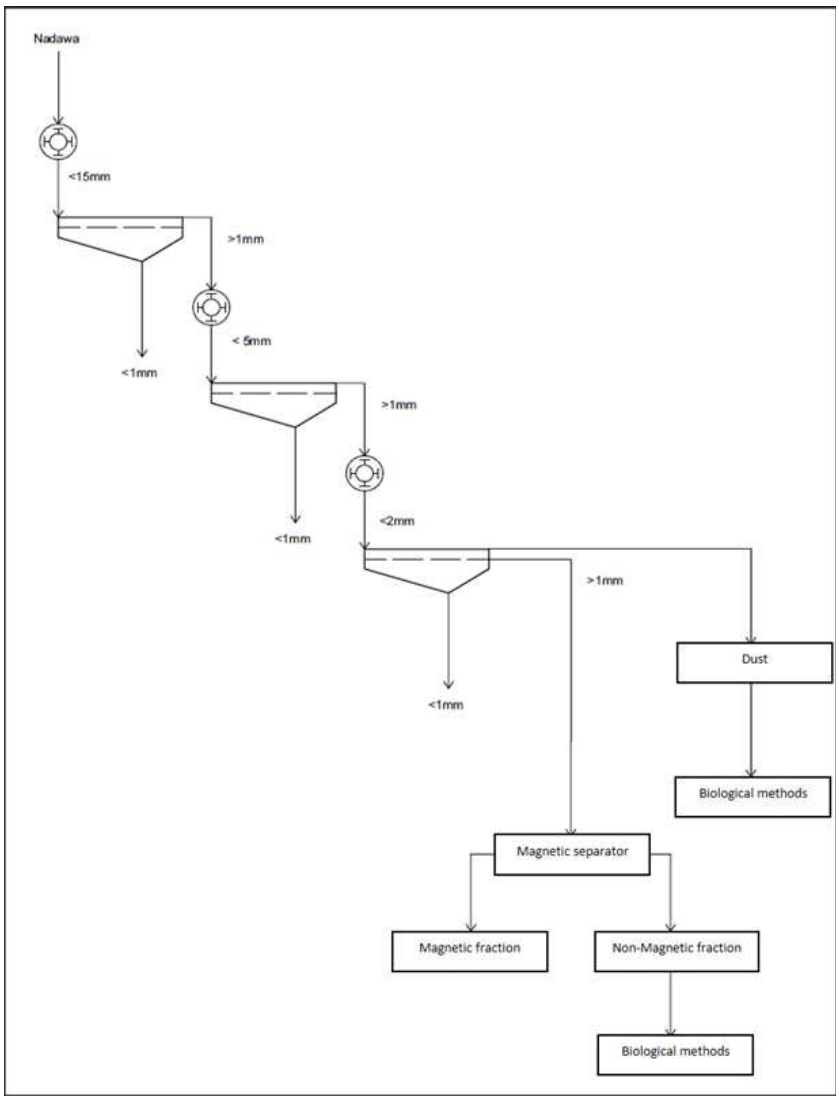

Fig. 2. Diagram of processing printed circuits board of a mobile phone
Rys. 2. Schemat przeróbki płytek drukowanych pochodzących z telefonów komórkowych

Tab. 1. Chemical analysis for metal content of WPCBs
Tab. 1. Patka. 1. Analiza chemiczna zawartości metali w WPCB

Metal type	Metal content (%w/w)
Al	0,36
Cu	14,76
Ag	0,0012

containing 200ml of Sabouraud broth BTL-P-0133 with no WPCBs was carried out. After 10 days of cultivation, the obtained mycelium was separated from the medium, then the medium was filtered in order to remove the remains of mycelium and spores. WPCBs with the concentration of 2.5% w/v was added to the obtained medium and the suspension obtained was left for 15 days. During a given period of the process, samples of the leaching solution were taken at selected intervals in order to analyse the metal content.

Results and discussion

Mechanism of fungal bioleaching

Bioleaching of metals by mould fungus usually involves indirect process consisting of bioproduction of organic acids, amino acids, and other metabolites. The metabolites produce dissolve (mobilise) metals in the two processes: the first is displacing metal ions from the solid matrix with hydrogen ions (acidolysis) and the second involves forming soluble metal and chelate complexes and their stabilisation in solution (complexolysis) (Xia M. et al., 2018; Işıldara A. et al., 2019). The dissociation and complexation reactions of organic acids that occur during the bioleaching process are presented below (Faraji F. et al., 2018):

Citric acid dissociation and complexation:

$$C_6H_8O_7 \rightarrow C_6H_7O_7^- + H^+ \quad (pK_{a1} = 3{,}09)$$
$$C_6H_7O_7^- \rightarrow C_6H_6O_7^{2-} + H^+ \quad (pK_{a1} = 4{,}75)$$
$$C_6H_6O_7^{2-} \rightarrow C_6H_5O_7^{3-} + H^+ \quad (pK_{a1} = 6{,}40)$$
$$n[C_6H_7O_7^-] + M^{n+} \rightarrow M[C_6H_7O_7]_n$$
$$n\left[C_6H_6O_7^{2-}\right] + 2M^{n+} \rightarrow M_2[C_6H_6O_7]_n$$
$$n\left[C_6H_5O_7^{3-}\right] + 3M^{n+} \rightarrow M_3[C_6H_5O_7]_n$$

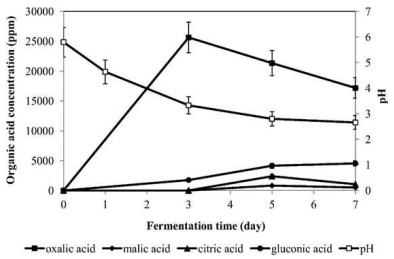

Fig. 3. Kinetics of production of organic acids. (Rasoulnia P., Mousavi S.M., 2016)
Rys. 3. Kinetyka tworzenia kwasów organicznych (Rasoulnia P., Mousavi S.M., 2016)

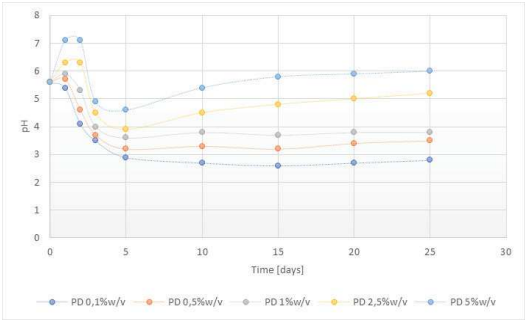

Fig. 4. Alteration in pH within the 25st days of one step bioleaching by A. niger (T=28°C; 120 rpm speed)
Rys. 4. Zmiany odczynu podczas 25 dni jednostopniowego procesu bioługowania z wykorzystaniem A. niger (T=28°C; 120 rpm speed)

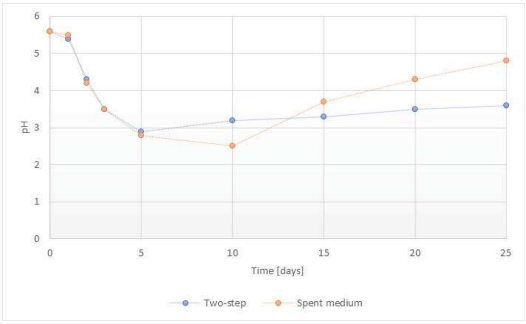

Fig. 5. Alteration in pH within the 25st days of two step/spent medium bioleaching by A. niger (T=28°C; 120 rpm speed; PD =2,5% w/w)
Rys. 5. Zmiany odczynu podczas 25 dni dwustopniowego/pośredniego procesu bioługowania z wykorzystaniem A. niger (T=28°C; 120 rpm speed; PD =2,5% w/w)

Oxalic acid dissociation and complexation:

$$C_2H_2O_4 \rightarrow C_2HO_4^- + H^+ \quad (pK_{a1} = 1{,}25)$$
$$C_2HO_4^- \rightarrow {C_2O_4}^{2-} + H^+ \quad (pK_{a1} = 4{,}14)$$
$$n[C_2HO_4^-] + M^{n+} \rightarrow M[C_2HO_4]_n$$
$$n\left[{C_2O_4}^{2-}\right] + 2M^{n+} \rightarrow M_2[C_2O_4]_n$$

Gluconic acid dissociation and complexation:

$$C_6H_{12}O_7 \rightarrow C_6H_{11}O_7^- + H^+ \quad (pK_a = 3{,}86)$$
$$n[C_6H_{12}O_7^-] + M^{n+} \rightarrow M[C_6H_{12}O_7]_n$$

Malic acid dissociation and complexation:

$$C_4H_6O_5 \rightarrow C_6H_5O_5^- + H^+ \quad (pK_{a1} = 3{,}40)$$
$$C_6H_5O_5^- \rightarrow {C_6H_4O_5}^{2-} + H^+ \quad (pK_{a1} = 5{,}11)$$
$$n[C_6H_5O_5^-] + M^{n+} \rightarrow M[C_6H_5O_5]_n$$
$$n\left[{C_6H_4O_5}^{2-}\right] + 2M^{n+} \rightarrow M_2[C_6H_4O_5]_n$$

All organic acids mentioned above play a key role in the process of leaching metals from PCBs. However, citric acid and oxalic acid are of the most importance. This is due to the fact that citric acid has the greatest ability for complexation while oxalic acid is one of the strongest acids. Hydrogen ions of acids release (mobilise) metal ions and metal chelation stabilise them in the solution.

It should be noted that compared to bacterial leaching, fungal bioleaching has several advantages, namely:

- ability to grow at higher pH, making it more suitable for biological leaching of alkaline material
- usually shorter bioleaching process time
- chelation of metal ions with metabolites (organic acids) reduced their toxicity (Xia M. et al., 2018).

Organic acids production kinetics

During the first three days of Aspergillus niger mycelium growth, accelerated production of oxalic acid occurs, which is gradually decreasing, but at the end of the seventh day there is still a significant amount of it in the medium. Such a relationship occurs because in the early days of fungal growth, the pH of the medium is higher and oxalic acid secretion occurs mainly when pH values are above 4.

As the concentration of oxalic acid in the medium decreases, gluconic acid production increases. Gluconic acid secretion occurs at pH values lower than values for oxalic acid

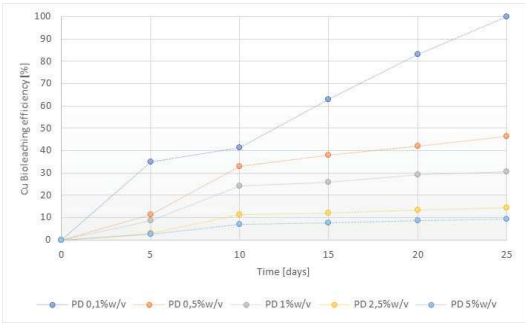

Fig. 6. Bioleaching efficiency of Cu as a function of time; one-step bioleaching, Aspergillus niger, T=28°C; 120 rpm speed
Rys. 6. Skuteczność bioługowania Cu w funkcji czasu; jednostopniowe bioługowanie, Aspergillus niger, T=28°C; 120 rpm speed

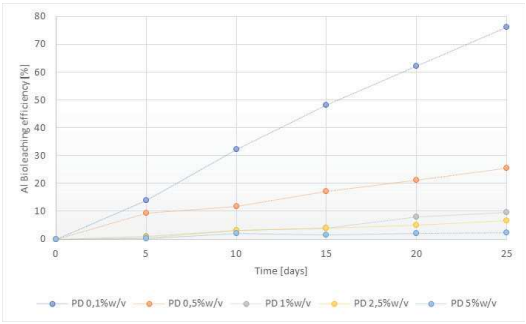

Fig. 7. Bioleaching efficiency of Al as a function of time; one-step bioleaching, Aspergillus niger, T=28°C; 120 rpm speed
Rys. 7. Skuteczność bioługowania Al w funkcji czasu; jednostopniowe bioługowanie, Aspergillus niger, T=28°C; 120 rpm speed

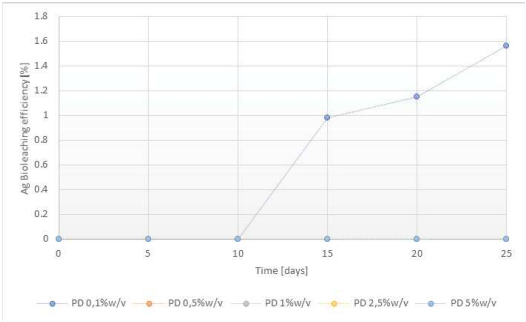

Fig. 8. Bioleaching efficiency of Ag as a function of time; one-step bioleaching, Aspergillus niger, T=28°C; 120 rpm speed
Rys. 8. Skuteczność bioługowania Ag w funkcji czasu; jednostopniowe bioługowanie, Aspergillus niger, T=28°C; 120 rpm speed

production but higher than those for citric acid production. Thus, as long as the pH of the medium is maintained within the acceptable range, gluconic acid will be produced to a sufficient degree and then its production will gradually be stopped.

Citric acid production carried out by Aspergillus niger does not occur in the exponential growth phase, but in secondary productivity, which is characterised by a reduced growth rate and low external pH. Therefore, taking into consideration the first days of mycelium growth, the production of citric acid is negligible compared to other organic acids. In case of the longer growth period and keeping the low pH of the medium, citric acid would probably be the main metabolite present in the medium as it reduces the concentration of oxalic acid present in the medium by using it in the Krebs cycle.

After three days of mycelium growth, the production of malic acid commences gradually, but its concentration is much lower compared to other present organic acids. (Rasoulnia P., Mousavi S.M., 2016).

However, it should be noted that the presence of metals in the medium may affect the kinetics of organic acid production. According to published research, in one and two-step bioleaching process, the presence of Cu ions can interrupt citric acid production, the existence of Mn ions in the medium can cause gluconic acid production (Wu H.Y., Ting, Y.P., 2006; Xu T.J. et al., 2014) while oxalic acid concentration may be higher in the presence of heavy metals (Santhiya D., Ting Y.P., 2005).

Changes in pH of culture medium during bioleaching

The aqueous solution is one of the most important factors in bioleaching process. It is related to the kinetics of organic acids production, which have a decisive influence on leaching of metals from WPCBs. The following Graphs 1–2 illustrate the changes in pH over time using different bioleaching methods.

The graphs show trends in pH changes depending on the bioleaching model. In all three cases, one might spot an initial

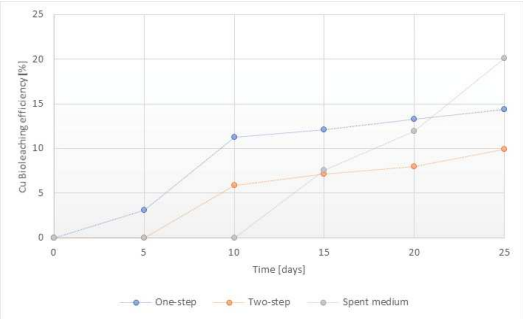

Fig. 9. Bioleaching efficiency of Cu as a function of time of a) one step at PD of 2%w/v, b) two step at PD of 2%w/v and c) spent medium at PD of 2%w/v (Aspergillus niger, T=28°C; 120 rpm speed)

Rys. 9. Skuteczność bioługowania Cu w funkcji czasu; a) jednostopniowe bioługowanie, b) dwustopniowe bioługowanie c) bioługowanie pośrednie (Aspergillus niger, T=28°C; 120 rpm speed)

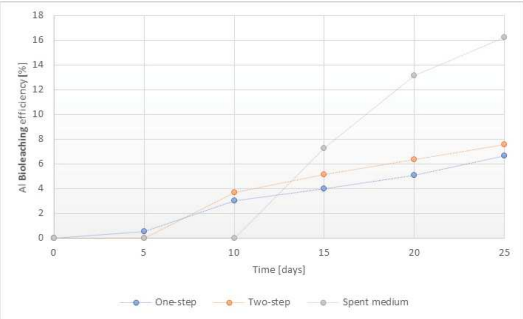

Fig. 10. Bioleaching efficiency of Al as a function of time a) one step at PD of 2%w/v, b) two step at PD of 2%w/v and c) spent medium at PD of 2%w/v (Aspergillus niger, T=28°C; 120 rpm speed)

Rys. 10. Skuteczność bioługowania Al w funkcji czasu; a) jednostopniowe bioługowanie, b) dwustopniowe bioługowanie c) bioługowanie pośrednie (Aspergillus niger, T=28°C; 120 rpm speed)

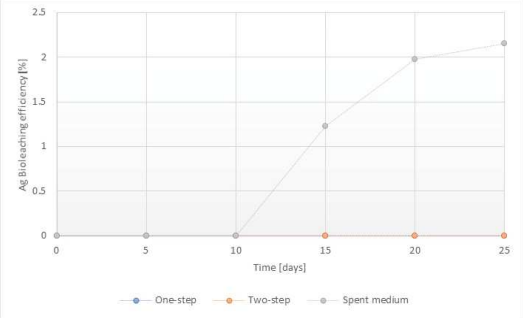

Fig. 11. Bioleaching efficiency of Ag as a function of time a) one step at PD of 2%w/v, b) two step at PD of 2%w/v and c) spent medium at PD of 2%w/v (Aspergillus niger, T=28°C; 120 rpm speed)

Rys. 11 Skuteczność bioługowania Ag w funkcji czasu; a) jednostopniowe bioługowanie, b) dwustopniowe bioługowanie c) bioługowanie „spent medium" (Aspergillus niger, T=28°C; 120 rpm speed)

sharp drop in pH over a period of 5 days, which corresponds to a phase of exponential growth of mycelium and production and dissociation of organic acids. (Faraji F. et al., 2017; Kolenčík M. et al., 2013). After that time, changes in pH were dependent on the presence and concentration of PD, but general trends were still visible. The downward trend is associated with the formation of organic acids. The upward trend, on the other hand, is caused either by the use of organic acids in the leaching process or by inhibition/disturbance of mycelium activity by metals (Horeh et al., 2016; Xia M. et al., 2018). What is more, according to Amiri et al. (2012), the 10th day of incubation is the end of the active growth phase and is followed by a decrease in the concentration of citric acid in the medium, which might be associated with the resorption of organic acids by mycelium.

Metals recovery at different fungal bioleaching approaches

Another crucial parameter affecting the efficiency of bioleaching is the concentration of metals in suspension (PD). It has been observed that a high content of WPCBs in the suspension has a negative effect on microorganisms and reduces the rate of bioleaching. This is due to the toxicity of metals and/or the insufficient amount of oxygen in the case of bacterial biomass, which inhibits the bioleaching rate (Zhu N. et al. 2011; Ilyas S. et al., 2010; Ilyas S. et al., 2013; Yang Y. et al., 2014). Graphs below illustrate the recovery of metals over time using different bioleaching methods.

The analysis of the results shows a clear relationship between metal recovery and PD and the chosen bioleaching method. Graphs 3–5 show that as the PD increases, metal recovery in the one-step bioleaching approach decreases. One

might explain it by assuming that the presence of a high concentration of toxic metals has an inhibitory effect on organisms. The growth and activity of the inoculum can be delayed or even inhibited, which is limited to a one-step bioleaching process with low PD. In addition, concerning a one-step process, it is challenging to provide optimal conditions for both the growth of organisms and metal extraction, which may also contribute to lower metal recovery in this process (Faraji F. et al., 2018; Kolenčík M. et al., 2013; Ilyas S. et al., 2013).

The analysis of shows the highest metal recovery to occur in the spent medium bioleaching method. In this case, contact of mycelium with toxic metals is completely eliminated. This results in higher metal extraction over less amount of time and the possibility to carry out the process with higher PD. However, concerning this method, the content of organic acids in the used medium depends on the conditions in which the culture was carried out. This may result in effective leaching of only some metals, depending on the concentration of the relevant organic acid (Faraji F. et al., 2018).

In the case of two-step bioleaching process, the effect of toxic metals on mycelium in the initial growth phase is eliminated. Mycelium can grow in optimal conditions until the first metabolites are produces. This should result in higher metal extraction over less amount of time and the possibility to carry out the process with higher PD. The analysis of the graphs shows that in this method, the recovery is higher concerning Al, but lower concerning Cu. This might be due to the low concentration of oxalic acid, which is most effective concerning mobilisation and retaining of Cu in a solution, which is due to its ability to produce insoluble oxalates. (Cui, H., Anderson, C.G., 2016; Santhiya, D., Ting, Y.P.,2005; Faraji F. et al., 2018).

Conclusions

Technological innovations and increased demand for electronic devices resulted in production of more and more waste with high metal content. This means that e-waste recycling is not only beneficial for waste neutralisation but also for the recovery of metals, including precious and rare-earth metals. Traditional e-waste recycling techniques, namely pyrometallurgical and hydrometallurgical techniques, are eco-unfriendly, energy-intensive, and noneconomic. This posed a challenge of using biotechnology to process e-waste and recover metals in an economical and environmentally friendly way.

The research presented in the article aimed at assessing the usefulness of the biotechnological method for leaching of selected metals from e-waste. The results indicate that it is possible to mobilise metals from the WPCBs using microorganisms such as Aspergillus niger fungi. For some elements, complete solubilisation has been achieved. However, it can be noted that in order to recover as much metal as possible, one should carry out a two-step or spent medium bioleaching process. This reduces the inhibitory effect of metals on mycelium growth and the production of metabolites which provides greater control over the process. Nonetheless, the application of biotechnological methods on an industrial scale requires further research in order to eliminate discrepancies concerning process parameters present in the literature and optimise those parameters.

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Bioługowanie wybranych metali z odpadowych obwodów drukowanych z wykorzystaniem grzybów mikroskopowych

Rosnący popyt na metale nieżelazne w ciągu ostatnich stuleci wywierał stałą presję na zasoby naturalne. W przypadku wielu ważnych i najczęściej używanych surowców na wyczerpaniu są już złoża łatwo dostępne i wysokiej jakości. Bogate, wtórne źródło metali stanowią odpady elektryczne i elektroniczne, których ilość w UE corocznie wzrasta. W celu zwiększenia efektywności gospodarowania zasobami i przyczynienia się do gospodarki o obiegu zamkniętym niezbędne jest usprawnienie przetwarzania i recyklingu urządzeń elektronicznych (WEEE) pod koniec ich życia. Dobór odpowiedniej metody przetwarzania tych odpadów jest bardzo ważny ze względu na złożony i różnorodny, pod względem materiałowym, skład zużytego WEEE. Szczególne znaczenie zarówno pod kątem środowiskowym jak i gospodarczym mają odpadowe obwody drukowane (WPCBs) stanowiące 3-5% wagowych WEEE. W artykule badano odzysk Cu, Ag i Al z WPCBs z wykorzystaniem przemysłowego szczepu grzyba pleśniowego Aspergillus niger. Proces bioługowania prowadzono 3 metodami (jednoetapowy, dwuetapowy, z wykorzystaniem pożywki zawierającej metabolity w inkubatorze z wytrząsaniem w zależności od czasu kontaktu oraz różnych gęstości zawiesiny.
Badania przedstawione w artykule miały na celu ocenę przydatności metody biotechnologicznej do ługowania wybranych metali z odpadów elektronicznych. Wyniki wskazują, że jest możliwe mobilizowanie metali z WPCBs przy użyciu mikroorganizmów.

Słowa kluczowe: *bioługowanie metali, odpadowe płyty drukowane, grzyby*