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LCD panels bioleaching with pure and mixed culture of *Acidithiobacillus*

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Abstract: The influence of pure and mixed culture of *A. ferrooxidans* and *A. tiooxidans* as well as different pulp density (1 and 2%) of LCD panels on the In and Sn bioleaching efficiency was investigated. Pulp density is one of the factors affecting the metals extraction efficiency during biological leaching. It has been shown that lower pulp density results in higher indium and tin dissolution. The *A. ferrooxidans* bioleaching system showed better metal extraction results than *A. thiooxidans*, especially for tin, indicating the special role of iron and *A. ferrooxidans* in tin recovery. The highest leaching rate of both indium (94.7%) and tin (98.2%) was obtained using iron and sulfur medium inoculated with mixed bacteria and a pulp density of 1% w/v.

Keywords: bioleaching, Aciditiooxidans, ITO, indium, tin, LCD panels, recovery

1. Introduction

Indium belongs to the group of critical raw materials (CRM - Critical Raw Materials) as one of the elements with a high supply risk for the European Union (EU) and a significant impact on the development of strategic EU sectors (renewable energy, electric mobility, aviation and digital technologies) (COM, 2020). Most of the indium (over 55%) is used to produce Indium-Tin-Oxide (ITO) (Uebersachar et al., 2017), a component of LCD (Liquid-Crystal Display) touch screens, televisions, computers and other electronic components, which are produced in hundreds of millions of pieces annually. Depending on the manufacturer, the indium content in LCD screens ranges from 100-300 mg In/kg of glass, while in primary sources, such as sphalerite or chalcopyrite ores, it ranges from 10 to 20 mg In/kg (Yang, 2012; Zhang et al., 2015). Despite extensive research, a complete LCD recycling cycle has not been developed, indium is not recovered and the recycling of used LCD screens has become a real challenge. The process of indium and tin recovery from LCD panels has been studied by many different methods of dissolution, extraction, separation, and purification (Zhang et al., 2015; Amato and Belonchini, 2018), such as the leaching process (Rochetti et al., 2015; Qin et al., 2021), solvent extraction (Ruan et al., 2012; Pereira et al., 2018) selective precipitation or a method of vacuum-chlorinated separation (Ma et al., 2012).

Chemical leaching is characterized by a high recovery efficiency in a short time (e.g. 100% recovery of indium in a one-step 2M H_2SO_4 leaching process, 80 °C for 10 minutes) (Rocchetti et al., 2015), however, requires strong acids and/or high temperature. The search for effective solutions, apart from classic mechanical, hydrometallurgical and pyrometallurgical methods, also includes bio-

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hydrometallurgical methods, using the potential of microorganisms. Biological methods are an attractive alternative, they do not require extreme parameters and offer low cost and environmentally friendly benefits (Vestola et al., 2010; Willner et al., 2015; Sedlakova-Kadukova et al., 2017). Microorganisms are capable of transforming many metals found in many valence states by catalysing redox reactions. Bacterial strains most commonly used in bioleaching on a laboratory scale and in industrial applications belong to the genus *Acidithiobacillus*. They can obtain the oxidizing energy both reduced sulfur compounds and the ferrous ion. There is an increasing amount of new results on the utilization of acidophilic microorganisms in the bioleaching of various types of metal-bearing wastes (Printed Circuit Boards - PCBs, Ni-Cd batteries, Li-ion batteries, spent refinery catalysts) with high efficiency of metals bioleaching (> 90%) (Sedlakova-Kadukova et al., 2017; Willner and Fornalczyk, 2013; Nagar et al., 2021), including valuable In extraction from LCD (Willner et al., 2018; Jowkar et al., 2018; Xie et al., 2019; Rezaei et al., 2018).

Previously, both pure and mixed cultures of *Acidithiobacillus tiooxidans* and *Acidithiobacillus ferrooxidans* were used in the recovery of indium, which derive energy from the chemical oxidation of elemental sulfur (they produce sulfuric acid) or oxidation of iron (II) (Jowkar et al., 2018; Xie et al., 2019). Indium bioleaching has been shown to have a good metal recovery potential from spent LCD compared to chemical leaching. Indium was recovered from waste LCD at 100% in 15 days, with adapted *A. thiooxidans* (LCD density 1.6% w/v) (Jowkar et al., 2018) or even shorter - in 6 days (Xie et al., 2019) using adapted sulfur *Acidithiobacillus* (LCD density 1.5% w/v) while the chemical leaching was 74% and 8% respectively. It was also found that 100% efficiency was achieved when adapted *A. ferrooxidans* (LCD density 2.5% w/v, 10 days) was used, however Xie et al. found that they did not obtain any leaching of indium in the presence of *A. ferrooxidans*. In systems with non-adapted *A. ferrooxidans* bacteria, their growth was stopped (due to the toxicity of the LCD powder) and the recovery of indium did not exceed 10% (Rezaei et al., 2018). Adaptation is a key factor in ensuring the effectiveness of indium leaching, however, reports by various authors indicate that high efficiency was obtained both in the presence of pure bacteria that derive energy from the biooxidation of inorganic compounds containing reduced S as well as Fe^{2+}. It has not been clearly established which bacterial strains and oxidizing agents play a leading role in indium bioleaching.

Previous literature analysing the possibility of recovering metals from LCD material using bacterial leaching is significantly limited. The works relating to bioleaching (Jowkar et al., 2018; Rezaei et al., 2018, Xie et al., 2019) did not take into account the presence of tin, which in 10% as SnO_2 is a component of ITO (another 90% is In_2O_3) in the LCD material. Jowkar et al. focused on the recovery In and Sr from LCD screen sources from laptops, where Sr constituted 0.2% of the material. Other researchers (Rezaei et al., 2018, Xie et al., 2019) analysed only the possibility of In recovery. There is therefore no publications for Sn, bound in the ITO material with In. The knowledge about the behaviour of tin is particularly important from the point of view of the possibility of its co-dissolution in the biooxidation of ITO components and the effective separation of this metal in further purification processes. Our previous work (Willner et al., 2018) with mixed bacteria of *A. thiooxidans* and *A. ferrooxidans* showed that presence of ferrous/ferric ions plays the important role in tin extraction from LCD. Tin was leached with an efficiency of more than 90% in both biological and control systems from the 9K medium, while in H_2SO_4 solution, the tin efficiency did not exceed 10%. It was also confirmed that 55% of indium was transferred to the solutions within 35 days (Willner et al, 2018). The aim of this study was to assess the effectiveness of In and Sn bioleaching with Fe or S substrates through acidophilic activity of bacteria. Various pure and mixed culture of *A. ferrooxidans* and *A. thiooxidans* strain, pulp density, (1%, 2% w/v) and contact time were tested.

2. Materials and methods

2.1. Material preparation

The LCD screens were sourced from a variety of used cell phones in the market. The disassembly of the LCD panels was manual, which allowed for the separation of the research material from the remaining components of the phones. The LCD panel is an element containing two layers of glass substrates filled with a conductive ITO layer, coloured pigments and thin film transistors. The outer sides of the insulating glass are covered with a layer of polarizing foils that adhere tightly to the glass, the separation of which is significantly difficult. One of the ways to separate the film from the glass is by mechanical processing. However, due to the significant loss of glass material during grinding, which does not separate from the polarizing film, an effective wet separation method was used applying NaOH solution (Ueberschaar et al., 2017). The glass panels were cut into pieces approximately 1x1 cm and placed in a beaker with 2M NaOH for 20 hours at ambient temperature. After this time, all material was filtered off, washed and dried at 70 °C for 1h. In this way, two material fractions were obtained: glass separated from ITO and the material of the polarizing foil with the remainder of non-separated glass (Fig. 1). To reduce glass loss, the material was washed again with NaOH solution recovered from the first washing phase at 70 °C for 1 hour. In this way, a foil-free glass fraction was obtained, which, after washing and drying, was ground by a knife mill (ChemLand FW135, Poland). The ground materials were sieved in an electromagnetic sieve shaker (Multi-Serw-Morek LPzE-2e, Poland) equipped with sieves (2.0-0.1 mm). Material with a grinding of <0.1 mm was used for further bioleaching tests, as shown in Fig. 1c.

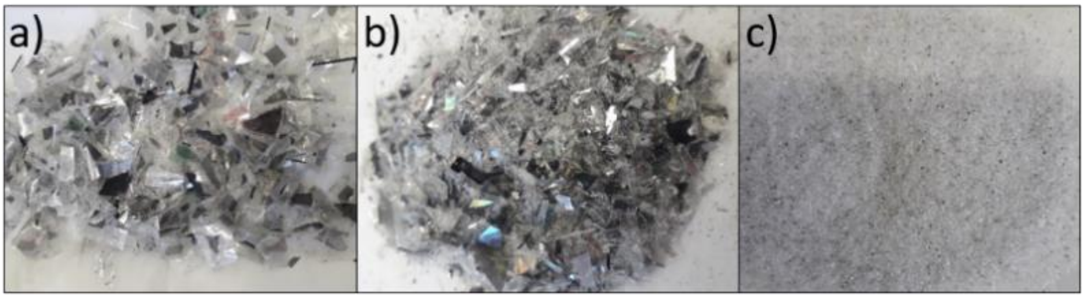

Fig. 1. LCD material obtained after treatment of 2M NaOH: a) Polarizing film contaminated with glass; b) separated fraction of glass from ITO, c) ground fraction of LCD glass < 0.1 mm

2.2. Bioleaching

Biological leaching was performed with the pure strain and a mixed culture of *A. ferrooxidans* and *A. thiooxidans*. Pure cultures of the *Acidithiobacillus ferrooxidans* strain SmolnikLC and A. *thiooxidans* strain SmolnikF were obtained from the Institute of Geotechnics of the Slovak Academy of Sciences in Košice. Both bacteria were recovered from the mine's acid drainage from a copper mine near Smolnik in Slovakia. The experiments were carried out in the media 9K and Waksman and Joffe for A. *ferrooxidans* and *A. thiooxidans*, respectively, and with a medium consisting of (g/L): $(NH_4)_2SO_4$-2.0; KCl-0.1; $MgSO_4{\cdot}7H_2O$-0.25; KH_2PO_4-0.25; $FeSO_4{\cdot}7H_2O$-44.2, S^0-10, for mixed *A. ferrooxidans* and *A. thiooxidans*.

Due to the limited efficiency of In and Sn bioleaching at S/L ratio 2.5% (w/v) (Willner et al., 2018), we reduced the pulp density to 1% and 2% (w/v). Erlenmeyer flasks containing ground LCD glass samples and a suitable medium for pure and mixed bacteria were inoculated with 10% (v/v) of the bacteria strain (initial pH = 2.0-2.2) and kept at 30 °C in a thermostat during experiments. Bioleaching tests lasting 35 days were carried out and in parallel, control tests were carried out under sterile conditions in a medium without bacteria. All biological and control experiments were performed in a least in duplicate. Regular measurements of pH, oxidation-reduction potential (Eh) and metals concentration were performed. The adaptation process with indium and tin compounds was carried out for pure *A. ferrooxidans* and *A. thiooxidans* for 10 weeks. Adaptation was carried in 190 mL of nutrient medium with addition 10 ml of pure culture of *A. ferrooxidans* or *A. thiooxidans*, with a dose of ground LCD material (≤ 0.1 g) added every 3 days.

5 ml of effluent were regularly collected and filtered for analysis of indium and tin concentrations in chemical and biological leaching solutions. The metal content of each filtrate was determined by Microwave Plasma Emission Spectroscopy (MPAES) (Agilent MP-AES 4200). Regular measurements of the oxidation-reduction potential (Eh) (CP-505, electrode ERPt-13, Hydromet, Poland) and pH (pH-meter CP-505, electrode ERH-11 Hydromet) were performed. In order to determine the qualitative composition of the LCD material during the experiment, a scanning electron microscope (SEM) equipped with Hitachi S 4200 and the method of microanalysis (EDS) was performed. Table 1 and Fig. 2 show the characteristics of MPAES and SEM-EDS of the ground LCD material. The glass sample

consists mainly of amorphous silicon (Toache et al., 2020; Willner et al., 2021), indium and tin additives and other elements (e.g. Cu, Pb, Al, Sr, Ni, Cr, Mn) described in more detail in the previous publication (Willner et al., 2021).

Table 1. Metal content in the ground LCD material

Metal	In	Sn	Cu	Pb	Al
Content, %	0.0906	0.0250	0.0097	0.0009	0.3460

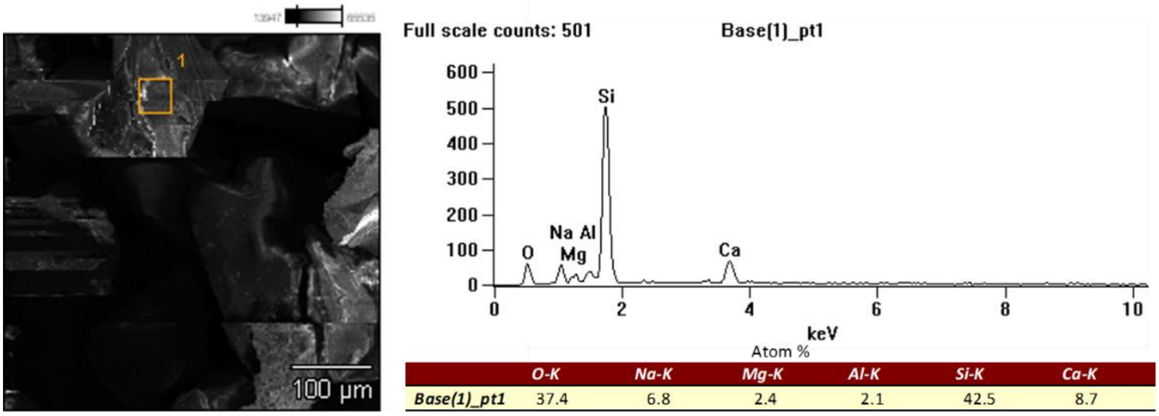

Fig. 2. SEM-EDS analysis of the 0.1 mm LCD glass fraction

3. Results and discussion

3.1. Bioleaching with pure *A. ferrooxidans* and *A. thiooxidans* - pH and ORP effect

Bioleaching of indium and tin from LCD panels by pure culture of *A. ferrooxidans*, A. *thiooxidans* and their mixture were tested for 35 days. During the bioleaching process, the microbial oxidation of ferrous ions to ferric ions and elemental sulphur to sulfuric acid occurs as follows:

$$S^0 + H_2O + 1.5O_2 \xrightarrow{A.thiooxidans} H_2SO_4 \quad (1)$$

$$2Fe^{2+} + {}^1/_2 O_2 + 2H^+ \xrightarrow{A.ferrooxidans} 2Fe^{3+} + H_2O \quad (2)$$

Fig. 3 shows the change in pH during bioleaching with pure bacteria and in control tests. The acidification of the leaching solutions and the decrease in pH are visible for both *A. ferrooxidans* and *A. thiooxidans* systems. In the environment with bacteria favoring the oxidation of S^0 to H_2SO_4 (reaction 1) the drop in pH is faster than in *A. ferrooxidans*, especially at a lower pulp density (1%), where the final pH value was 1.7. The acidification of the environment with *A. ferrooxidans* is mainly caused by the hydrolysis reactions and precipitation of iron (III) compounds in the form of jarosite (especially in the pH range from 1.8 to 2.7), where the precipitation of jarosite is an acid-forming reaction (Grishin et al., 1989):

$$3Fe^{3+} + X^+ + 2HSO_4^- + 6H_2O \rightarrow XFe_3(SO_4)_2(OH)_6 + 8H^+ \quad (3)$$

where X is a K^+, Na^+, $NH_4^{+,}$ or H_3O^+.

Apart from NH_4^+ (ions in the 9K medium), monovalent cations as Na^+, K^+, contained in the panels of thin-film transistor liquid crystal display (TFT-LCD) in a form of Na_2O, K_2O (Kae-Long et al., 2009) contribute to the formation of ferric hydroxyl salts, which was visible in leaching system in the form of yellow-brown phase. At the same time, the pH of the control samples ranged from 2.1 to 2.4 (Fig. 3b)

In the systems with *A. ferrooxidans*, the concentration of Fe^{3+} (reaction 2) increases with the progress of the reaction and the Eh potential increases also (Fig. 4). The growth of Eh is from around 400 mV to around 660 mV for pure A. *ferrooxidans*. However, the Fe^{2+} biooxidation process is slightly faster when the pulp density is 1%. Ferrous ions presented in bacterial leaching solutions are regenerated in reaction of biooxidation to Fe^{3+} catalysed by the bacteria. The Eh value in the control samples, in which Fe (II) oxidation occurs spontaneously under the influence of atmospheric oxygen, did not exceed 360 mV.

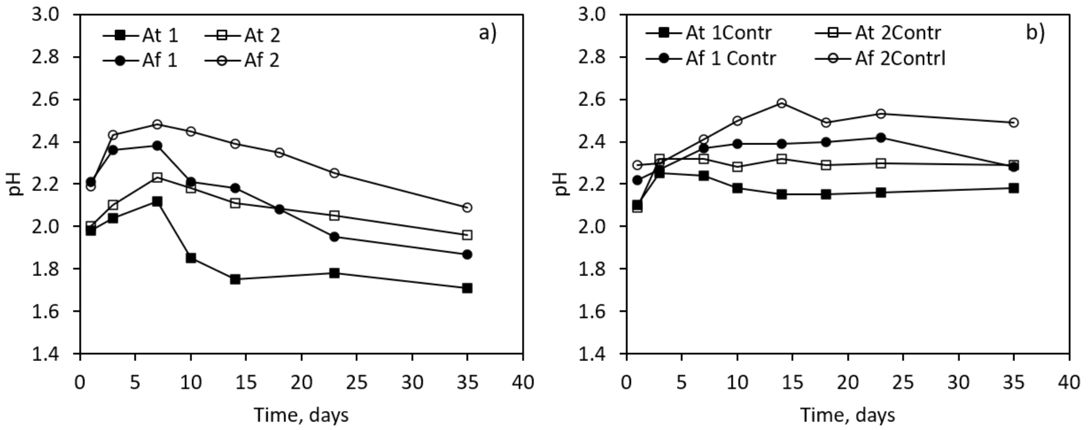

Fig. 3. Changes in the pH value over time and the diversity of pulp density (1% w/v and 2% w/v): a) pure *A. ferrooxidans* (Af) and *A. thiooxidans* (At); b) control samples

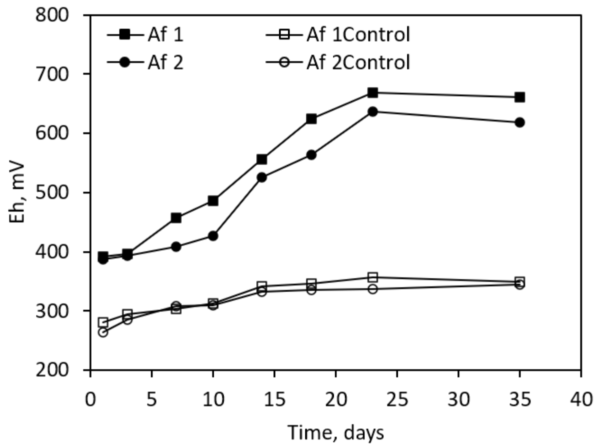

Fig. 4. Changes in Eh value over time and differences in pulp density (1% w/v and 2% w/v) with bacteria *A. ferrooxidans* and control samples

3.2. Effect of pulp density with pure bacteria

Our research concerns the influence of pulp density due to the importance of this factor. It is known that increasing the pulp density in the bacterial leaching process inhibits the kinetics and rate of metal extraction (Rezaei et al., 2018; Valix, 2017; Brandl et al., 2001). The results obtained so far clearly indicate that the high bio-recovery efficiency of In was obtained by using a pulp density in the range of 1-1.6% (w/v) (Jowkar et al., 2018; Rezaei et al., 2018, Xie et al, 2019). Increasing the pulp density to 2.5% significantly lowered the cell concentration of bacteria and biooxidation, reducing the recovery efficiency of this metal (Willner et al., 2018, Rezaei et al. 2018). Therefore, we reduce pulp density to the value: 1% and 2% w/v. Fig.s 5 and 6 compares the In and Sn bioleaching efficiency of LCD waste over 35 days with the variable pulp density for the systems with pure bacteria. Higher extraction efficiency of indium and tin was found when the lower pulp density was used - 1% (w/v) for both pure of *A. thiooxidans* and *A. ferrooxidans* cultures. Increasing the pulp density to 2% (w/v) significantly reduces the In and Sn extraction. Pulp density during bacterial adaptation is one of the key factors affecting the efficiency of indium extraction in the bioleaching process. The bacterial cell concentration was significantly reduced when pulp density increased from 0.5 w/v to 2.5% w/v, which limited cell multiplication, and as suggested by the author (Rezaei et al. 2018), there was a decrease in bacterial productivity by inactivating its enzymes due to the formation of metal ions of complexes with protein molecules in bacteria. Within 35 days, the highest indium leaching rate of 84.7% was obtained for pure *A. ferrooxidans*, while it was 61.3% in *A. thiooxidans* systems. An equally high degree of indium recovery (100%), obtained in shorter time (10 days) with adapted ferric bacteria, is presented in (Rezaei et al., 2018), however, these results do not coincide with the results of other authors (Xie et al., 2019; Jowkar et al., 2018), who bioleaching indium is attributed to *A. thiooxidans*. Xie et al. and Jowkar et al. achieved 100% leaching efficiency of indium in the presence of *A. thiooxidans* after 8 days and 15 days, respectively. H^+ ions have been recognized to play an important role in the dissolution of indium, however H^+ release is not the only factor in effective indium leaching (acid leaching cannot fully wash the indium out of the LCD powder) and bacterial involvement is necessary for a high leach efficiency (Xie et al., 2019). As suggested in the earlier work (Willner et al., 2018), iron has a significant influence on Sn extraction, which is reflected in the obtained results. Within 10 days, 83.9% and 47.3% of Sn were dissolved in *A. ferrooxidans* and *A. thiooxidans*, respectively (pulp density 1% w/v) – Fig. 5 and 6. Fe^{3+} ions in aqueous solutions of ferric chloride or ferric sulfate are used as an effective medium to completely detinning a scrap in just a few minutes at room temperature (C 22 B 25/06 patent). The reaction involving the tin bound to In in the LCD powder can be similar to *A. ferrooxidans* system based on oxide-reduction Fe^{2+}/Fe^{3+} (reaction 2), increased Fe^{3+} concentration in the solution dissolves out tin preferentially over time, which manifests itself faster extraction of Sn in relation to In (Fig. 5).

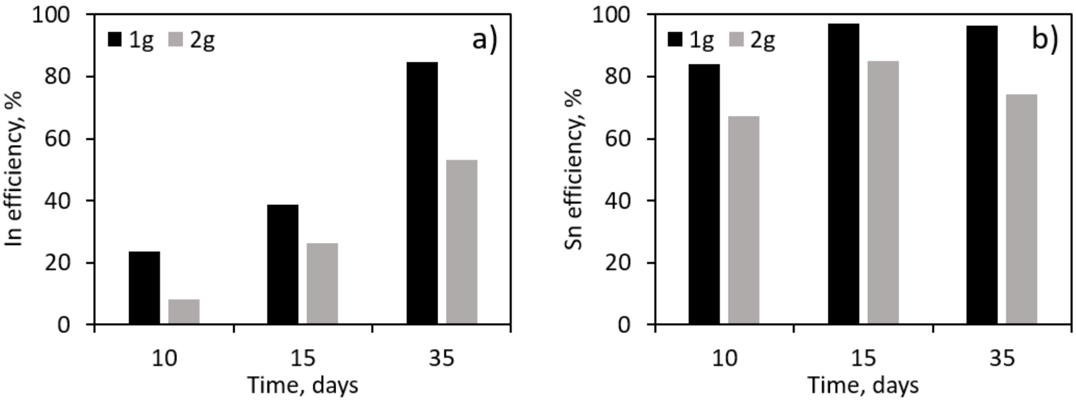

Fig. 5. Bioleaching efficiency of indium (a) and tin (b) with A. *ferrooxidans* for different pulp densities

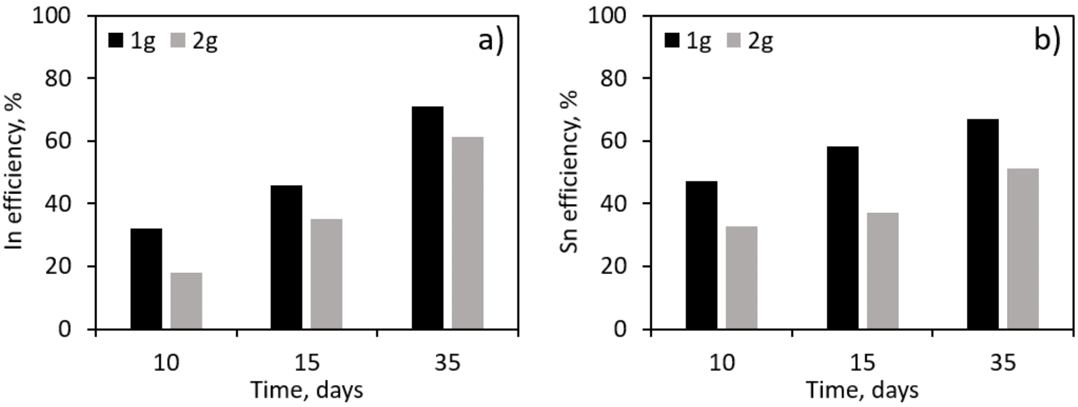

Fig. 6. Efficiency of indium (a) and tin (b) bioleaching with A. *thioxidantes* for different pulp densities

3.3. In and Sn bioleaching efficiency with mixed bacteria

Based on the obtained results of the research on the higher In and Sn bioleaching efficiency with the use of a lower density of LCD glass pulp, further studies with the participation of mixed bacteria were carried out for the pulp density 1% w/v case. The change in Eh and pH over time, shown in Fig. 7, is similar to that for pure strains, but the decrease in pH was faster, and the degree of acidification was lower. The visible initial increase in pH for both pure *A. ferrooxidans* and *A. thiooxidans* (Fig. 3a) and mixed bacteria (Fig. 7) is related to the chemically neutral nature of the tested material. LCD powder dissolved in distilled water showed a pH in the range of 7 (Vakilhap et al., 2016; Rezaei et al., 2018), which, in combination with the consumption of protons and oxygen by *A. ferrooxidans* and the time needed for S^0 oxidation for *A. thiooxidans*, results in an increase in pH in the initial phase of bioleaching. The decrease in pH is accompanied by an increase in Eh for 10 days in the range of 447-628 mV.

The leaching efficiency results are shown in Fig. 8. The tin leaching kinetics is much shorter - 87.3% of Sn was recovered within 10 days, while 33.2% of In was recovered. In the LCD powder, both metals exist as oxides, but the dissolution of Sn is faster, releasing this metal first. Therefore, it is possible that Sn can be partially separated from In in the initial phase of bioleaching. The maximum tin leaching was 98.1% (15th day). Indium bioleaching was gradual and the maximum amount of indium leached was 94.7% on day 35. Equally positive results of indium leaching with mixed bacteria were presented in (Xie et al, 2019), where the indium extraction effect was 78% (8 days). The reduction of pulp density from 2.5% (Willner et al., 2018) to 1% w/v significantly improved the efficiency of bioleaching of metals with mixed bacteria. The interaction of the two strains *A. ferrooxidans* and *A. thiooxidans* increases the dissolution kinetics of metals, ensuring faster acidification of the environment, and the Fe^{2+}/Fe^{3+} redox pair in the leaching system supports the biological oxidation and dissolution of metals, in particular tin. In the works of many authors, a higher efficiency of metal leaching with the use of mixed bacteria is cited. Iron and sulfur-oxidizing cultures are important for effective degradation of natural minerals and high metal extraction rates, e.g. copper from chalcopyrite (where ferric iron is an oxidizing agent; and to remove elemental sulfur formed on the mineral surface), (Fu et al., 2008; Qui et al., 2005; Akcil et al., 2007) but also for the recovery of valuable metals from urban mining waste such as electronic scrap (Ivanus, 2010; Ilyas et al., 2007).

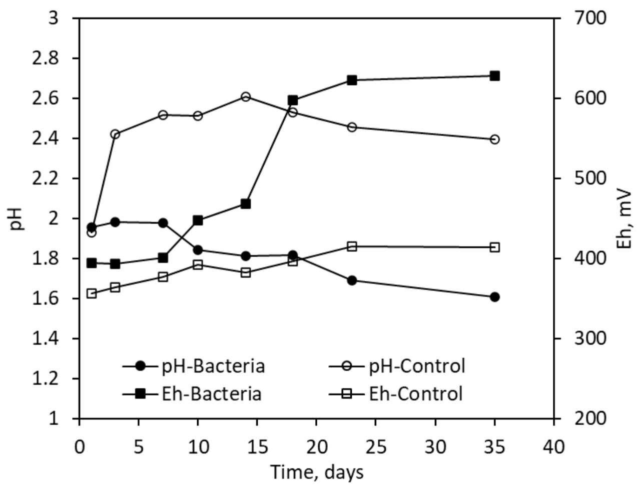

Fig. 7. Changes in pH and Eh values over time with mixed bacteria and control samples (pulp density 1% w/v).

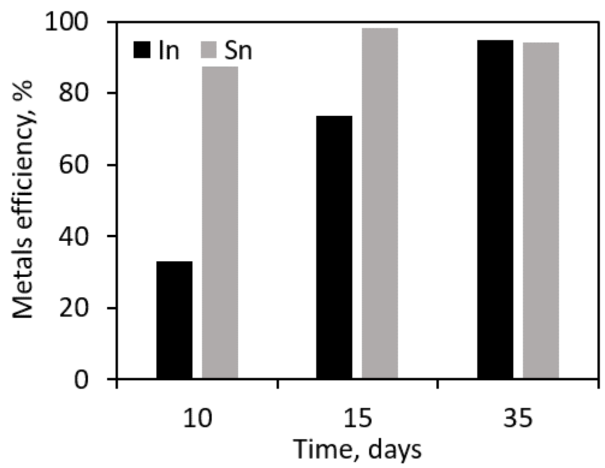

Fig. 8. Indium and tin bioleaching efficiency with mixed bacteria

4. Conclusions

Bioleaching of indium and tin from LCD panels was carried out with different efficiency depending on the pulp density (1% and 2% w/v) and the use of pure or mixed bacteria. When a higher pulp density was used, the kinetics and rates of metal extraction were inhibited when using the pure strain, therefore 1% w/v pulp density is an appropriate value for efficient bioleaching In and Sn from LCD material. Mixed cultures containing iron and sulfur oxidizing bacteria were more productive than a single pure culture. 98.2% of Sn and 94.7% of In were washed out with mixed bacteria (1% w/v), while for *A. ferrooxidans* and *A. thiooxidans* it was 84.7% for In, 97.3% for Sn and 71% for In and 66.9% for Sn, respectively. *A. ferrooxidans* and Fe^{3+} ions lead to a faster and more complete extraction of Sn than would be expected with *A. thiooxidans*. Therefore, the rapid dissolution of tin suggests the possibility of its successful separation and recovery at the beginning of bioleaching using more effective oxidizing agents such as iron oxidizing bacteria.

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