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Processing of Water Treatment Sludge by Bioleaching

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Received: 14 November 2020; Accepted: 9 December 2020; Published: 11 December 2020

Abstract: Biological metal leaching is a technology used in the mining and biohydrometallurgy industries where microorganisms mediate the dissolution of metals and semi-metals from mineral ores and concentrates. The technology also has great potential for various types of metal-rich waste. In this study, bioleaching was used for sludge from water treatment. In addition to checking the applicability of the process to such a substrate, the influence of experimental conditions on the effectiveness of bioleaching of metals with sludge from water treatment was also determined, including sample acidification, addition of elemental sulfur, incubation temperature, and *Acidithiobacillus thiooxidans*-isolated strain. The measurement of metal concentration and, on this basis, the determination of bioleaching efficiency, as well as pH and oxygen redox potential (ORP), was carried out during the experiment at the following time intervals: 3, 6, 9, 12 days. After the experiment was completed, a mass balance was also prepared. After the experiment, high efficiency of the process was obtained for the tested substrate. The effectiveness of the process for most metals was high (Ca 96.8%, Cr 92.6%, Cu 80.6%, Fe 95.6%, Mg 91%, Mn 99.5%, Ni 89.7%, Pb 99.5%, Zn 93%). Only lower values were obtained for Al (58.6%) and Cd (68.4%).

Keywords: bioleaching; metal recovery; *Acidithiobacillus thiooxidans*; sludge from water treatment

1. Introduction

Water processing sludge (WPS) is an indispensable byproduct of water treatment and is mainly generated during the coagulation of contaminants with aluminum or iron salts. Waste management of WPS is a problem mainly due to its chemical properties. Water sludge contains many contaminants such as toxic organic pollutants, metals, and pathogens [1,2]. Therefore, WPS disposal in agricultural use is difficult or even impossible. Even compost made from WPS does not meet expectations as a fertilizer. According to Marousek et al. [3], the use of WPS compost affects the behavior of phosphorus in soil. Iron present in the sludge turns the surrounding phosphorus into iron phosphates, which gradually become unavailable to plants and soil microorganisms as well [3].

Recycling in the construction industry by solidification is a popular method of utilization of water processing sludge. WPS could be successfully incorporated into the ceramic and building material [1]. Cremades at el. consider the applicability of WPS as co-material in the production of ceramic tiles [4]. Sludge solidification and neutralization may involve mixing with waste from lime production and stone polishing residues in order to produce clean construction materials like incombustible bricks and blocks [5]. Ling et al. investigated the possibility of using water processing sludge as a substitute for mineral clay [6]. The spectrum of WPS recycling is much broader and refers to the manufacture of materials such as lightweight aggregates [7], cement and concrete [8], and geopolymers [9].

From an ecological and economic point of view, water processing sludge can be applied in processes related to environmental engineering, especially wastewater treatment and waste management. By adding WPS to raw sewage, it is possible to accelerate the sedimentation of

particles and increase the reduction of suspended solids, including organic matter. Iron-based sludge and especially alum-based sludge could be a low-cost adsorbent to remove phosphorus (P) from raw wastewater [10,11]. Removal of suspended solids and phosphorus from wastewater occurs due to flocculation and/or physical sorption of contaminants on flocs generated during coagulation [12]. However, higher phosphorus removal effects were observed using WPS sludge produced using aluminum rather than iron coagulants. Al-based sludge usually has a higher total specific surface area and therefore a higher adsorption capacity [13].

Sludge regeneration through acidification and ultrafiltration allows for a more effective coagulating agent than in the case of direct WPS application [11,14]. It is also possible to use recovered coagulants for water treatment, using appropriate purification technology to remove dissolved organic carbon and trace metal contaminants [15].

Dosing WPS to reactors for biological wastewater treatment can be problematic due to toxicity and the amount of sludge [16]. Nevertheless, the use of sludge from water treatment for final precipitation after biological wastewater treatment, e.g., after using the Upflow anaerobic sludge blanket (UASB) reactor [17] or a conventional activated sludge method, may be considered [12,18].

The WPS application can also be beneficial in the treatment of sewage sludge. According to Li et al., the addition of alum sludge improved the sewage sludge dewatering [19]. This was possible because the aluminum sludge acted as a chemical conditioner by charge neutralization and adsorption, but also as a physical conditioner by its action as a structural agent [19].

Other possibilities of WPS application in environmental engineering processes have also been investigated. The addition of post-coagulation sludge in the anaerobic co-digestion process of food waste was conducive in shortening the lag phase and reducing the hydraulic retention time [20]. The limed water treatment sludge can be used as a low-cost adsorbent material for heavy metal removal due to the presence of various oxides in its components, mainly because of its large surface area [21]. Another example of ferric WPS application is as an efficient catalyst for the removal of volatile organic compounds (VOCs) such as propane and toluene [22].

Since the first stage of WPS regeneration requires acidification, it is possible to inoculate a sample of acidophilic bioleaching bacteria. Both chemical acidification and bioleaching processes will solubilize the majority of coagulant salts in the sludge [23].

Most of the revisions of bioleaching have concentrated on the use of a culture of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. The solubilization of metals from the substrate follows directly through the metabolism of leaching bacteria, or indirectly through the products of bacterial metabolism [24]. The bioleaching process has been an efficient and economical method for extracting, removing, and recovering valuable metals from minerals and municipal or industrial waste [25]. Bioleaching has been used for the removal of heavy metals from sewage sludge [26,27] and from dredged sediments [28,29] and for metal recovery from e-waste [30,31], ashes [32,33], and lithium batteries [34,35].

The main parameters determining the efficiency of bioleaching of metals from waste depend on microorganisms, growth substrates, temperature, type of bioreactor, and, above all, on the type and characteristics of the waste [29]. Therefore, each of the bioleaching applications will require exploratory tests to determine the usefulness of the method in the processing of selected wastes.

The substrate used in the research has so far been processed and neutralized by other processes. The used substrate could be applied as a coagulant but requires further processing to remove the ballast. The proposed solution aims at removing the ballast compounds in the substrate, especially metals. The novelty of the presented work is the application of the bioleaching process to sludge from water treatment. After the process, the produced biomass can be used for other purposes and the remaining liquid can be used as a coagulant.

The aim of the study was to determine the possibility of bioleaching, bacteria growth, and the bioleaching efficiency of selected heavy and light metals using sludge from water treatment as a culture medium. The influence of experimental conditions for the effectiveness of metal bioleaching with

sludge from water treatment was also determined, including sample acidification, elemental sulfur addition, incubation temperature, and the *Acidithiobacillus* genera inoculation. Measurement of metal concentration and, on this basis, the determination of bioleaching efficiency, as well as pH and redox potential, were carried out during the experiment at several time intervals: 3, 6, 9, 12 days. A mass balance was also made after the end of the experiment.

The target for the presented research will be the bioleaching process for the recovery of the coagulant. Post-culture liquids containing leached elements would be a substrate for the recovery process. However, this requires further experiments involving the separation of the solid phase (biomass) as well as the coagulant precipitation and purification stage (ultimately iron III sulfate). Another aspect is the possibility of using the resulting biomass as an acid fertilizer or plant conditioner.

2. Materials and Methods

2.1. Materials

The substrate (sludge) for this study was collected in the water treatment facility during the coagulation process. The underground water for treatment was taken from 5 intakes with a depth of 42 to 71 m from carbonate rocks. The maximum intake capacity was 1.000 m^3/h. The treatment station had the capacity to treat 750 cubic meters of water per hour. The equipment in the technological system of the water treatment plant included biofilters for denitrification, aerators, double-layer filters, carbon filters, an ozonation station, and a water tank. The sludge from denitrification and washings from the system were supplied to settling tanks. Thickened sludge from settling tanks was pumped to the sludge reservoir and then to the dewatering system. A coagulant in the form of a solution of iron (III) chloride was used in the process of water treatment. The coagulant was dosed to the installation of aerators and settling tanks. The tested water processing sludge (WPS) was a mixture of iron post-coagulation sludge and washings from biofilters and filters. WPS for this study was collected from the sludge reservoir. Table 1 shows the physicochemical characteristics of the collected sludge. The metal content (Al—aluminum, Ca—calcium, Cd—cadmium, Cr—chromium, Cu—copper, Fe—iron, Mg—magnesium, Mn—manganese, Ni—nickel, Pb—lead, Zn—zinc) of the sludge is presented in Table 2.

Table 1. Characteristics of collected sludge from water treatment.

Index	Units	Value
Dry solids (DS)	(g/L)	8.9 ± 0.1
Volatile solids (VS)	(g/L)	5.0 ± 0.1
pH	-	6.9 ± 0.3
Alkalinity	(mg $CaCO_3$/L)	571.7 ± 8.5
Total Kjeldahl nitrogen	(mg N/L)	721
Carbon content	(%DS)	27.80
Hydrogen content	(%DS)	4.41
Nitrogen content	(%DS)	4.84
Sulfur content	(%DS)	0.79
Dissolved organic carbon	(mg C/L)	73.9 ± 0.03%
Dissolved total Kjeldahl nitrogen	(mg N/L)	25.2
Dissolved ammonium nitrogen	(mg N-NH_4^+/L)	2.2

Table 2. The content of metals in the sludge collected for testing.

Metal	mg/kg	% DS
Al	1110.4	0.1110
Ca	26,703.7	2.6704
Cd	20.5	0.0021
Cr	446.8	0.0447
Cu	135.4	0.0135
Fe	242,474.7	24.2475
Mg	2978.1	0.2978
Mn	738.7	0.0739
Ni	30.1	0.0030
P	15,796.9	1.5797
Pb	152.7	0.0153
Zn	5042.0	0.5042

2.2. Inoculum Preparation

Culture of bioleaching bacteria was isolated from the WPS. In the cultivation of bioleaching bacteria, 9 K medium was used, composed of 3 g/L $(NH_4)_2SO_4$, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.5 g/L $K_2HPO_4 \cdot 3H_2O$, 0.1 g/L KCl, 0.01 g/L $Ca(NO_3)_2$, distilled water, and 10 g/L of elemental sulfur (S_0) as the energy source. The cultivation of the microorganisms was performed in 150 mL flasks using 100 mL of 9 K medium. Approximately 2 g of WPS was added to 100 mL of culture medium and the pH was adjusted to 2 with 5 N sulfuric acid. The flasks were shaken in an orbital shaker at 180 rpm at the optimum growth temperature of 30 °C for 7 days. The obtained bacteria were transferred to fresh medium three times, after which an inoculum for experiments was obtained. The inoculum parameters were as follows: pH 1.86 (±0.2), oxygen redox potential (ORP) = 560 mV (±65), total solids (TS_{IN}) and volatile solids (VS_{IN}) were 44.2 (0.3) and 37.6 g/L (0.3 g/L), respectively.

The species identification of the isolated microorganism intended for the bioleaching process was carried out in order to confirm the *A. thiooxidans* species of bacteria. For this purpose, PCR analysis with species-specific primers was used. Genomic DNA from the putative *A. thiooxidans* isolate was extracted using GeneMATRIX Gram Plus & Yeast Genomic DNA Purification Kit (Eurx, Gdansk, Poland). The PCR reaction was prepared as described previously (Zhu et al., 2012). PCR targeting the 16S rRNA genes was used to detect *A. thiooxidans* (primers Tt13: ATCACTGGGCGTAAAGGG and Tt2: AACCCAACATCTCACGACAC). Results confirmed the species of the isolated bacteria strain belonging to *A. thiooxidans* (Figure 1).

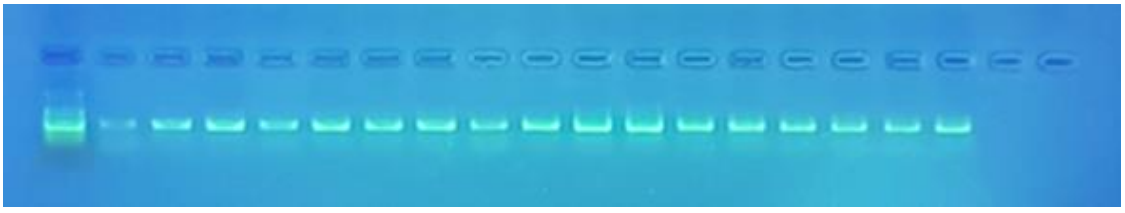

Figure 1. PCR results confirming *A. thiooxidans* bacteria. From left: first channel marker, second positive control; two last channels, negative control.

2.3. Bioleaching Experiments

The research was carried out in a 300 mL Erlenmeyer flask with an employed volume of 0.15 L. The volume of the WPS was 90% (*v*/*v*) and the active inoculum was 10% (*v*/*v*). The flasks were incubated in a shaker at 180 rpm and temperature of 30 and 20 °C, respectively, for 12 days. Elemental sulfur was added to the samples in the amount of 0.75 g (*w*/*v*) and 1.5 g (*w*/*v*). A control run without the addition of active inoculum (addition of distilled water) was also carried out to compare the results. The A–D combinations were the controls for the successive E–H combinations to test the suitability of

the inoculum. The studies were divided into 8 major combinations (A–H). Within each combination, samples were incubated at 20 and 30 °C (Table 3). The effectiveness of the bioleaching was assessed based on changes in the concentration of released trace elements (Al, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Zn) during the process. The assessment of culture conditions was based on changes in pH and redox potential. The analyses of the above-mentioned indices were carried out on days 3, 6, 9 and 12 of the process. A mass balance was also made after the end of the experiment.

Table 3. Research combinations for water processing sludge (WPS) bioleaching.

Treatment		pH Adjustment	Sulfur Addition, g	Incubation Temperature, °C
A20 A30	WPS (135 mL) + water (15 mL)	-	-	20 30
B20 B30	WPS (135 mL) + water (15 mL)	2.0	-	20 30
C20 C30	WPS (135 mL) + water (15 mL)	2.0	0.75	20 30
D20 D30	WPS (135 mL) + water (15 mL)	2.0	1.5	20 30
E20 E30	WPS (135 mL) + *A. thiooxidans* (15 mL)	-	-	20 30
F20 F30	WPS (135 mL) + *A. thiooxidans* (15 mL)	2.0	-	20 30
G20 G30	WPS (135 mL) + *A. thiooxidans* (15 mL)	2.0	0.75	20 30
H20 H30	WPS (135 mL) + *A. thiooxidans* (15 mL)	2.0	1.5	20 30

2.4. Analysis

Dry solids (DS), volatile solids (VS), total Kjeldahl nitrogen (TKN), ammonium nitrogen (NH_4^+-N), pH, and alkalinity were measured according to standard methods for the examination of water and wastewater [36]. Determination of selected elements using byoptical emission spectrometry with inductively excited plasma (ICP-OES) was carried out in accordance with PN-EN ISO 11885:209 [37]. Dissolved organic carbon (DOC) concentration was evaluated by the differential method using a TOC Analytik Jena multi N/C 3100 analyzer. For determination of the elementary content of carbon (C), hydrogen (H), nitrogen (N), and sulfur, the WPS LECO TruSpec device was used.

Prior to the measurement of selected trace elements TKN, NH_4^+-N, and TOC in the supernatant, the samples were centrifuged for 15 min at 11,200 rcf and filtered (0.45 µm, cellulose acetate). Samples for the analyses were collected after 3, 6, 9 and 12 days of the study. All the analyses were performed in triplicate. The results are presented in Figures 2–17 as the arithmetic means.

2.5. Statistical Analysis

Statistical analysis was carried out by the STATISTICA software (STATISTICA 13.3, TIBCO Software Inc., Palo Alto, CA, USA). The aim of the analysis was to determine differences in the efficiency of bioleaching depending on the combination of research (A20–H30). In the case of statistically important data ($p < 0.05$), Tukey's test for post-hoc analysis was used. The value bars in the figures marked with the same letter (a, b) are not statistically significant (no difference between combinations in the efficiency of the leaching of elements). The notations in the chart consisting of a letter and a combination name in parentheses are exceptions. These exceptions mean that there was a statistically significant difference.

3. Results and Discussion

3.1. Changes in pH and Oxidation-Reduction Potential during Study

The analysis of pH changes for individual combinations, regardless of the incubation temperature, was at a similar level, and the pH value ranged from 2 to 3. Only for combination A, the pH value was above 8 for both temperatures (Figures 2 and 3). Moreover, for the combinations B to H, a gradual decrease in the pH value was observed during the following days of the process. Only for combination E, the pH value on the third day of the test was approximately 5, after which a gradual decrease in pH below 2 was noted on the 12th day of the process. As reported by Zhang et al., 2009, lowering the pH by *A. thiooxidans* may be related to the use of sulfur for the biological production of H_2SO_4 [38]. However, a decrease in pH is usually specific for a bioleaching process with the addition of culture and the value is usually in the range of 2 to 3 [39]. An appropriate pH of the culture medium can enhance the activities of microbes in the bioleaching process. It also affects the solubilization of metals and the stability of metal ions in the liquid phase [29,33,40,41].

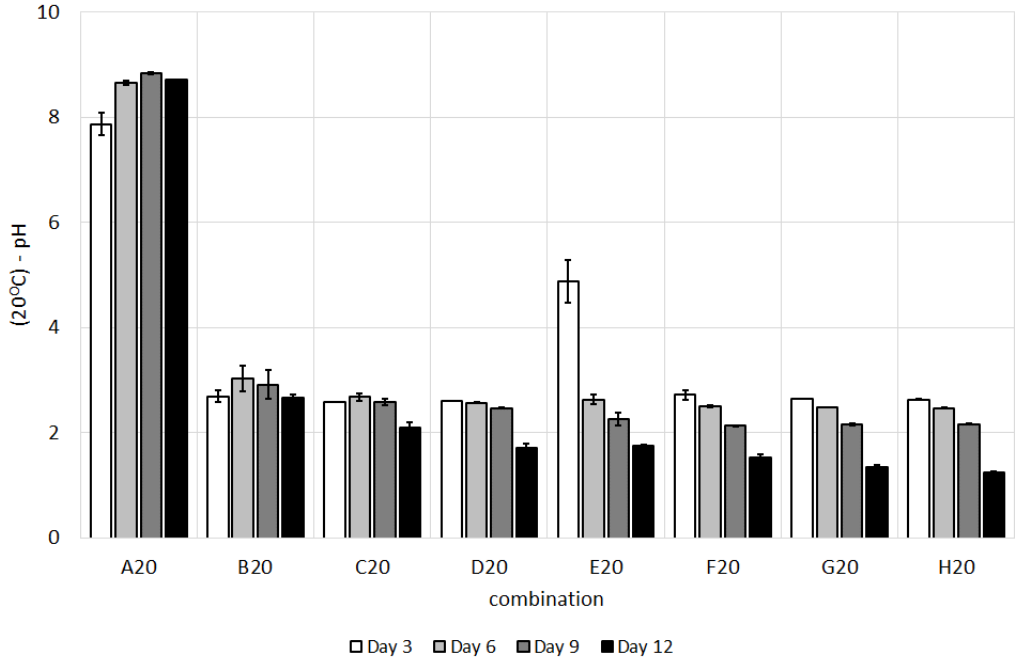

Figure 2. Changes in pH of bacteria culture samples incubated at 20 °C.

The ORP of all combinations increased from the start of the bioleaching process. From days 3 to 9, the ORP for combinations of A, E, G, and H increased, followed by a slight decrease on day 12 (Figures 4 and 5). At the end of the process, the ORP of the solutions bioleached at the temperature of 20 °C for the trials B, C, D, E was 600 mV; for the others, the ORP value was lower (400–500 mV). For bioleaching at 30 °C, the ORP at the end of the process was 600 mV for most combinations. According to the literature data, the release of metals increases with the decrease in the pH value and with a change in the redox conditions [42,43]. As reported by Kjeldsen et al., the critical factors for greater metal mobility are the decrease in pH value and the increase in redox potential [44]. Dissolution metals increased contaminant content by a reduction in pH and increase in ORP, as the high acidity can boost the dissolution of heavy metals and high ORP can dissociate the complexed sludge solids to release the heavy metals [45]. The high redox potential is suitable for the leaching of metals in an ore or solid waste. The culture medium redox potential increases with the bacterial activity, which benefits the bioleaching of the metals. However, the redox potential during bioleaching is not significantly affected by the amount of S_0 provided [29,33,40].

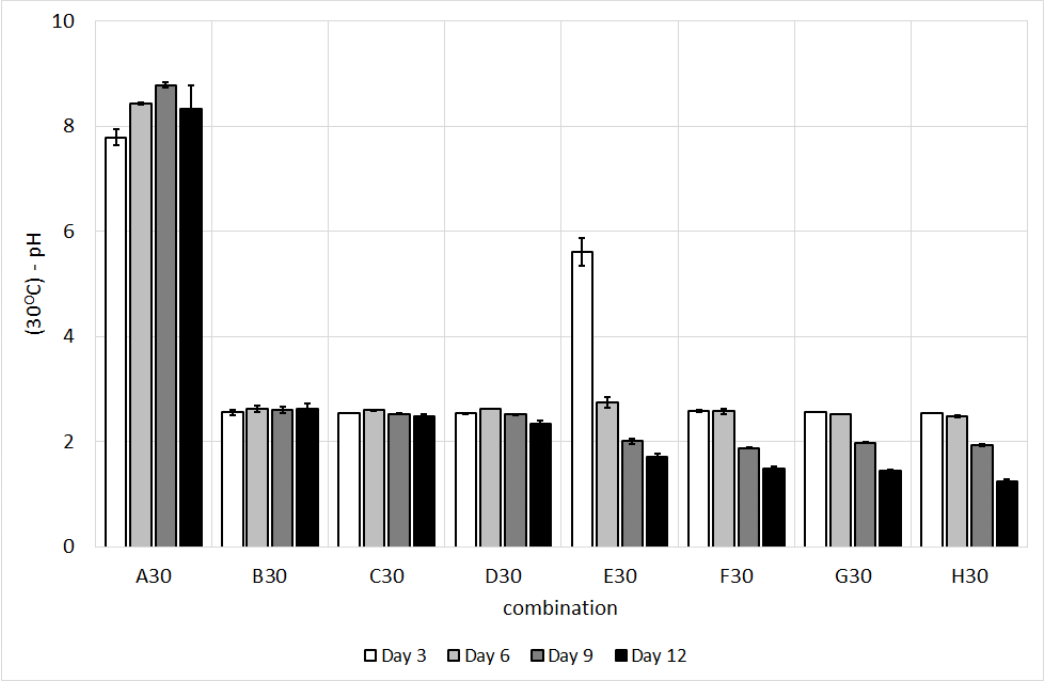

Figure 3. Changes in pH of bacteria culture samples incubated at 30 °C.

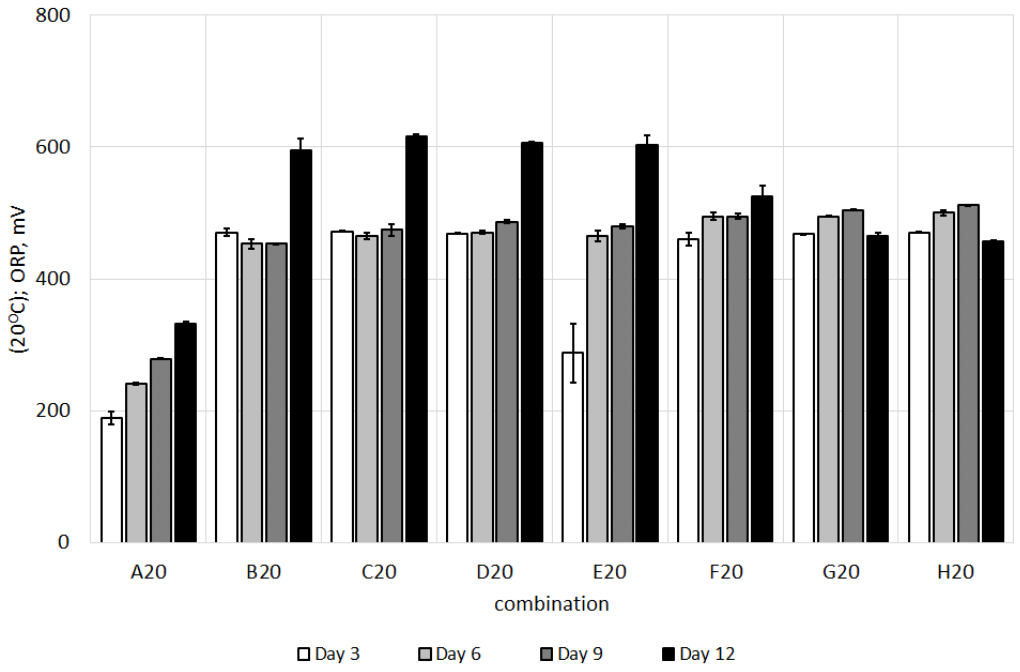

Figure 4. Oxidation reduction potential of samples incubated at 20 °C.

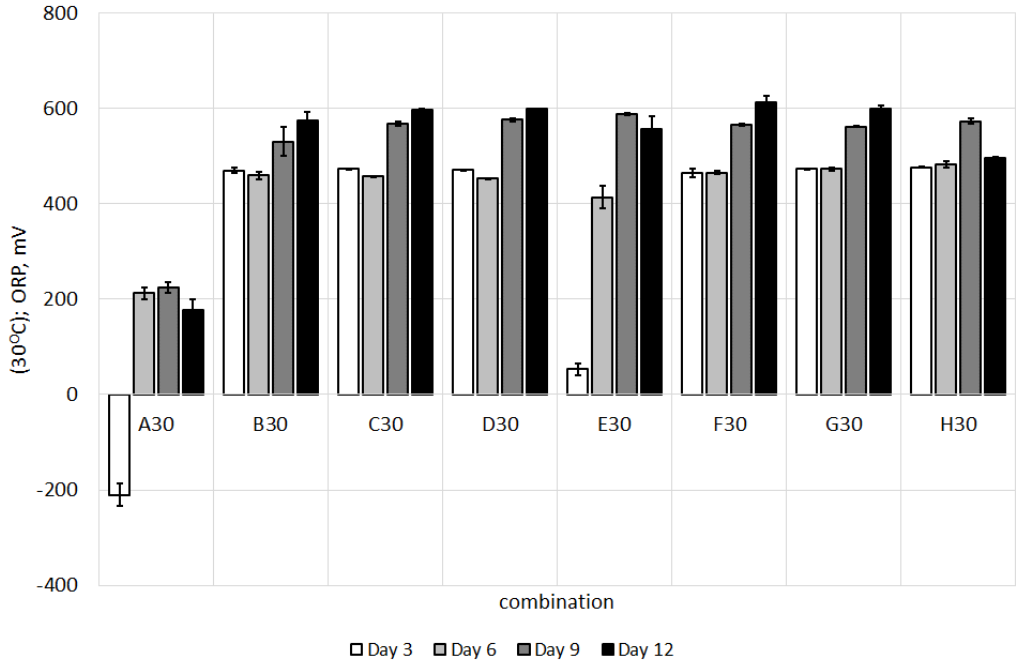

Figure 5. Oxidation reduction potential (ORP) of samples incubated at 30 °C.

3.2. The Removal of Heavy Metals during the Bioleaching

Bioleaching efficiency on particular days of the experiment—3, 6, 9, 12—was calculated relative to the primary concentration of trace elements. The results obtained for the individual metals are shown in Figures 6–16.

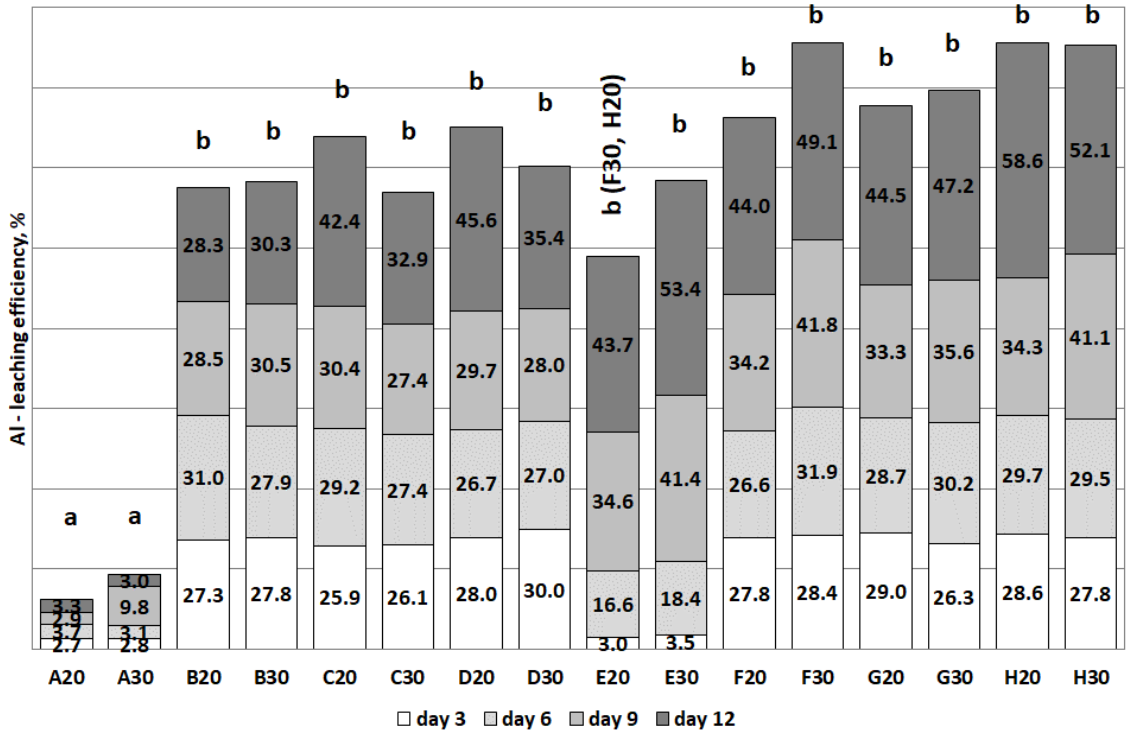

Figure 6. Bioleaching efficiency of Al during the experiment (Statistical groups a and b).

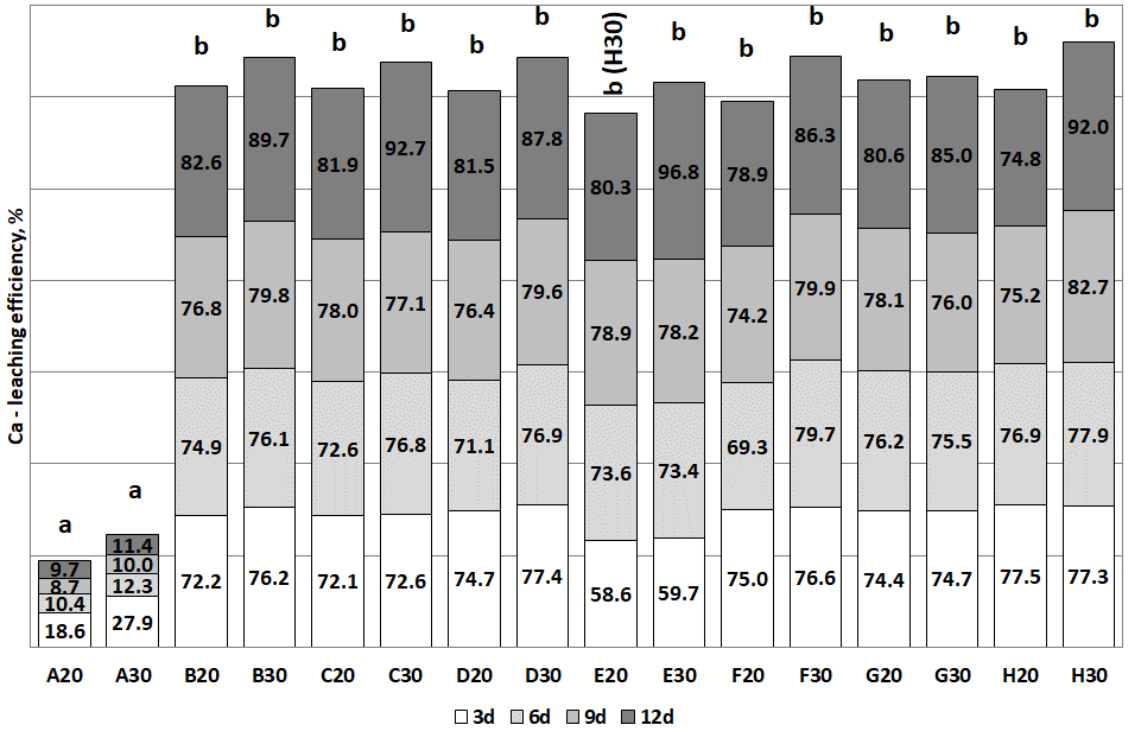

Figure 7. Bioleaching efficiency of Ca during the experiment (Statistical groups a and b).

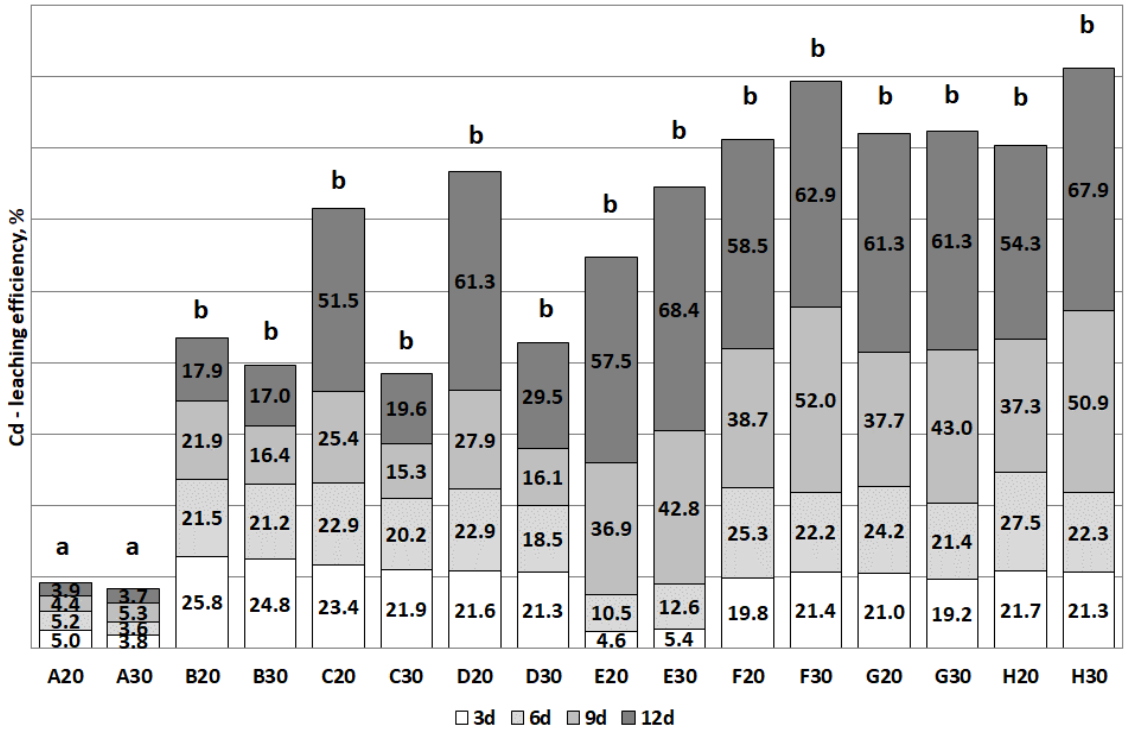

Figure 8. Bioleaching efficiency of Cd during the experiment (Statistical groups a and b).

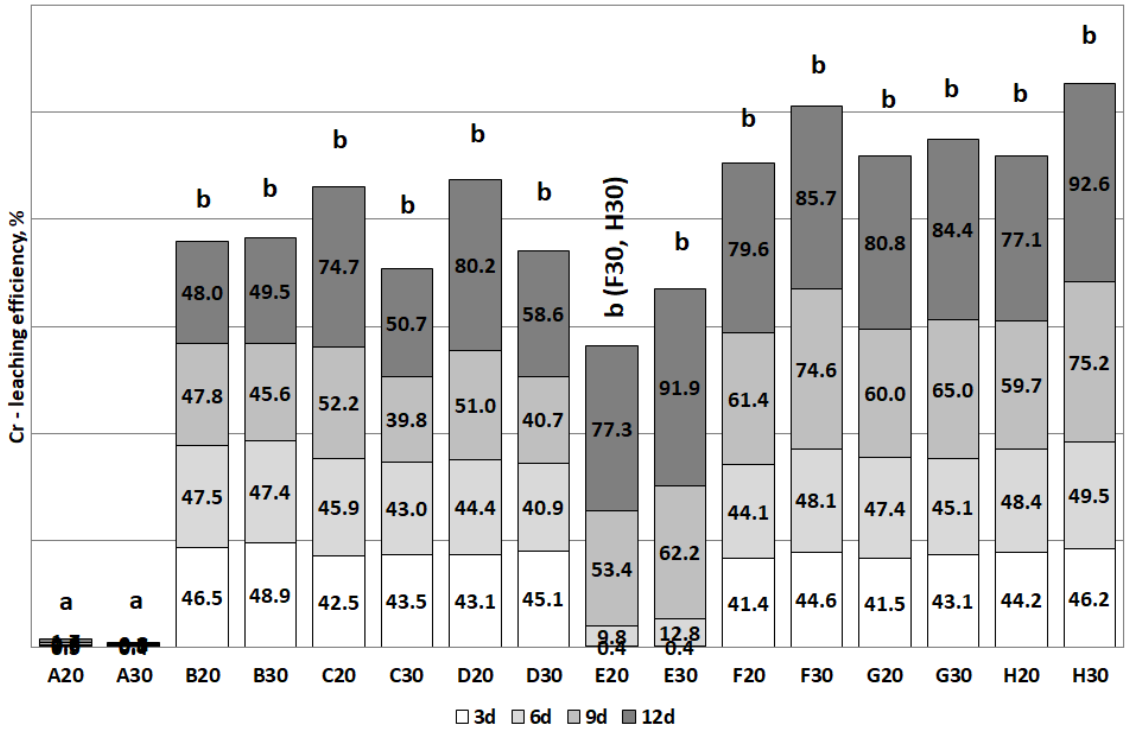

Figure 9. Bioleaching efficiency of Cr during the experiment (Statistical groups a and b).

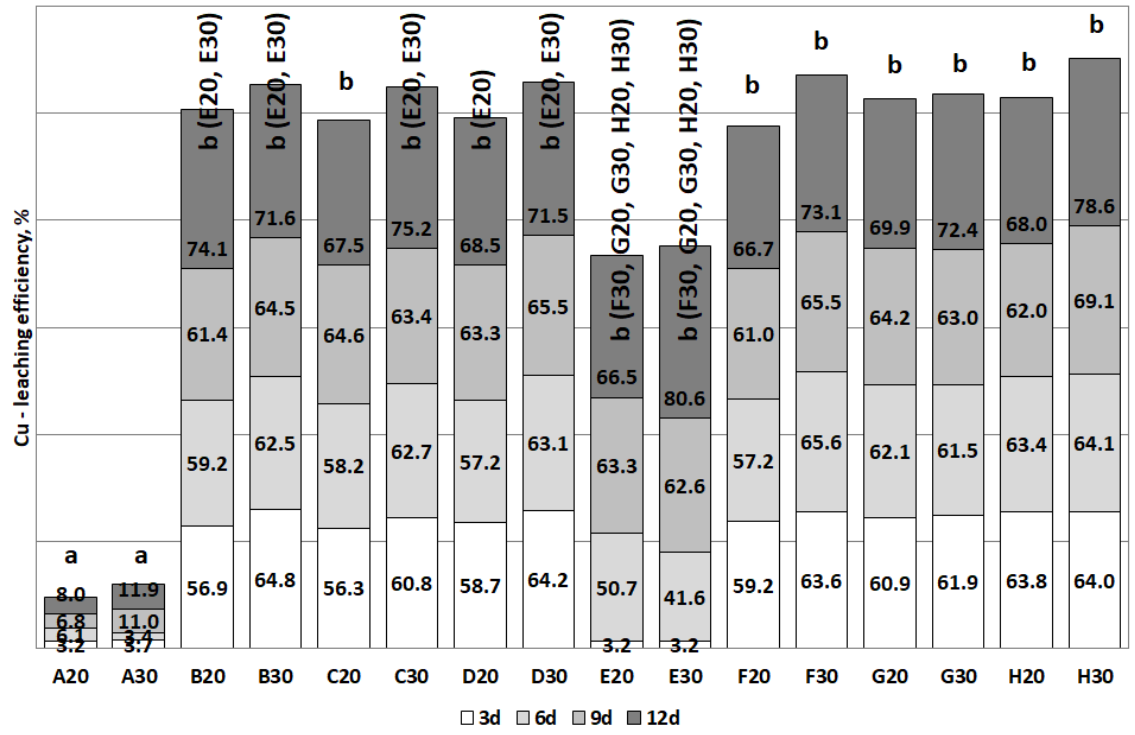

Figure 10. Bioleaching efficiency of Cu during the experiment (Statistical groups a and b).

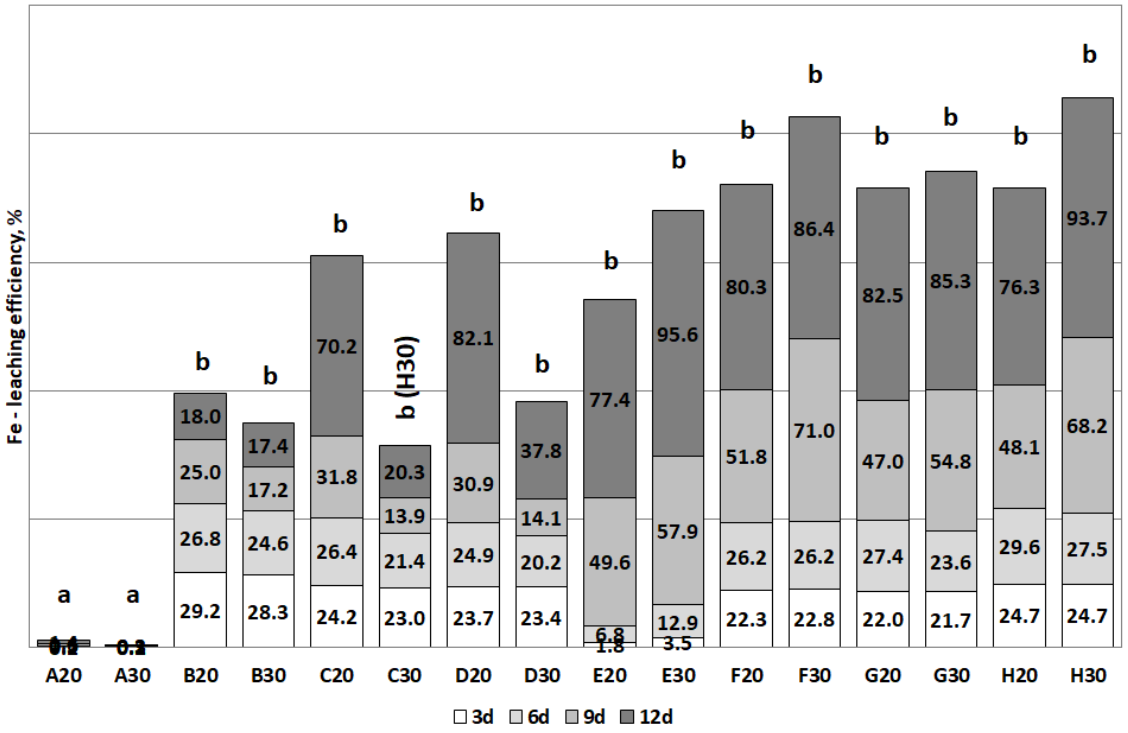

Figure 11. Bioleaching efficiency of Fe during the experiment (Statistical groups a and b).

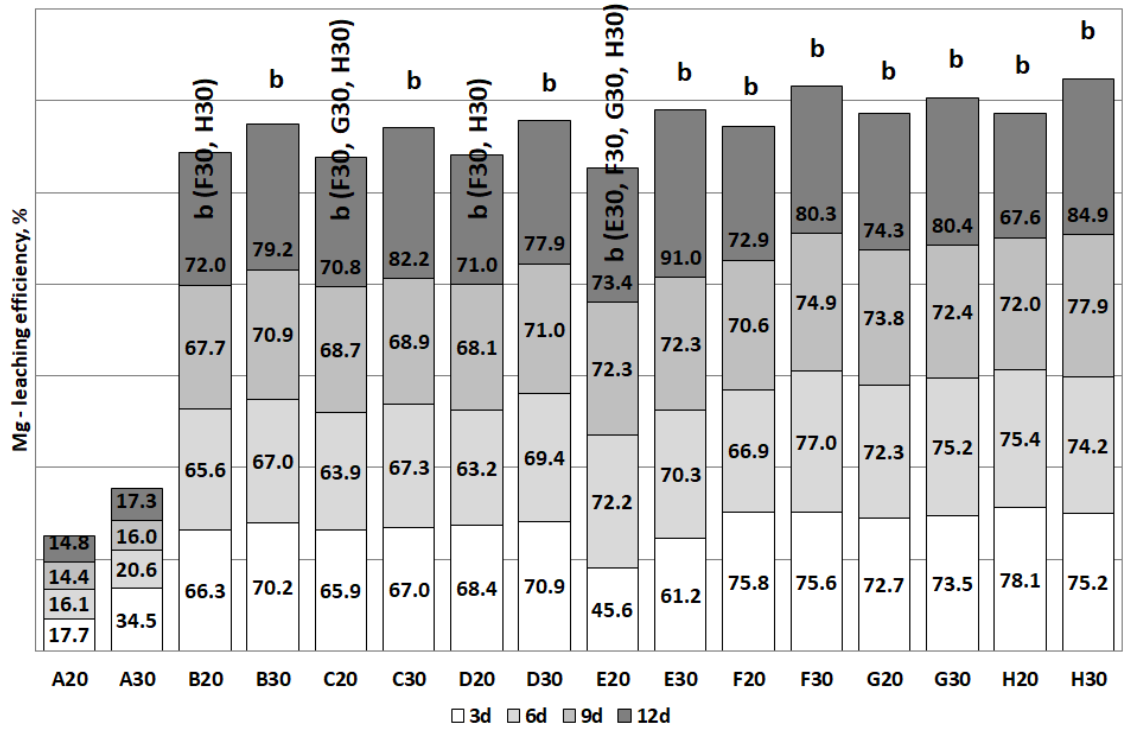

Figure 12. Bioleaching efficiency of Mg during the experiment (Statistical groups a and b).

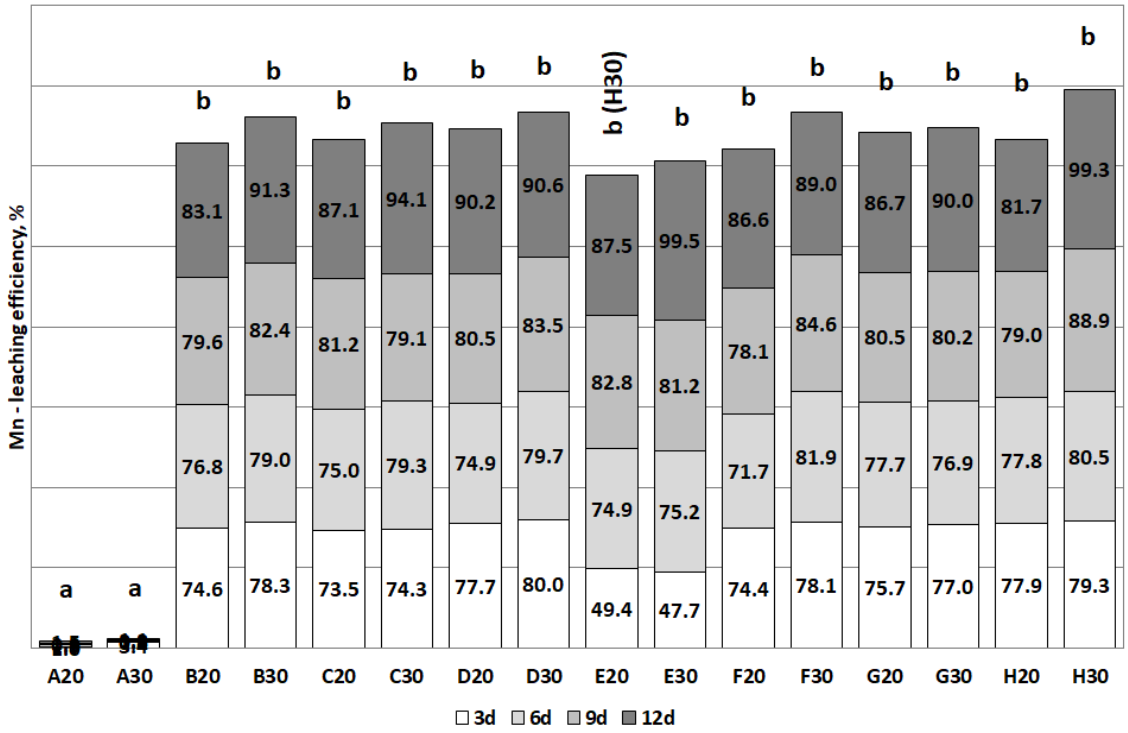

Figure 13. Bioleaching efficiency of Mn during the experiment (Statistical groups a and b).

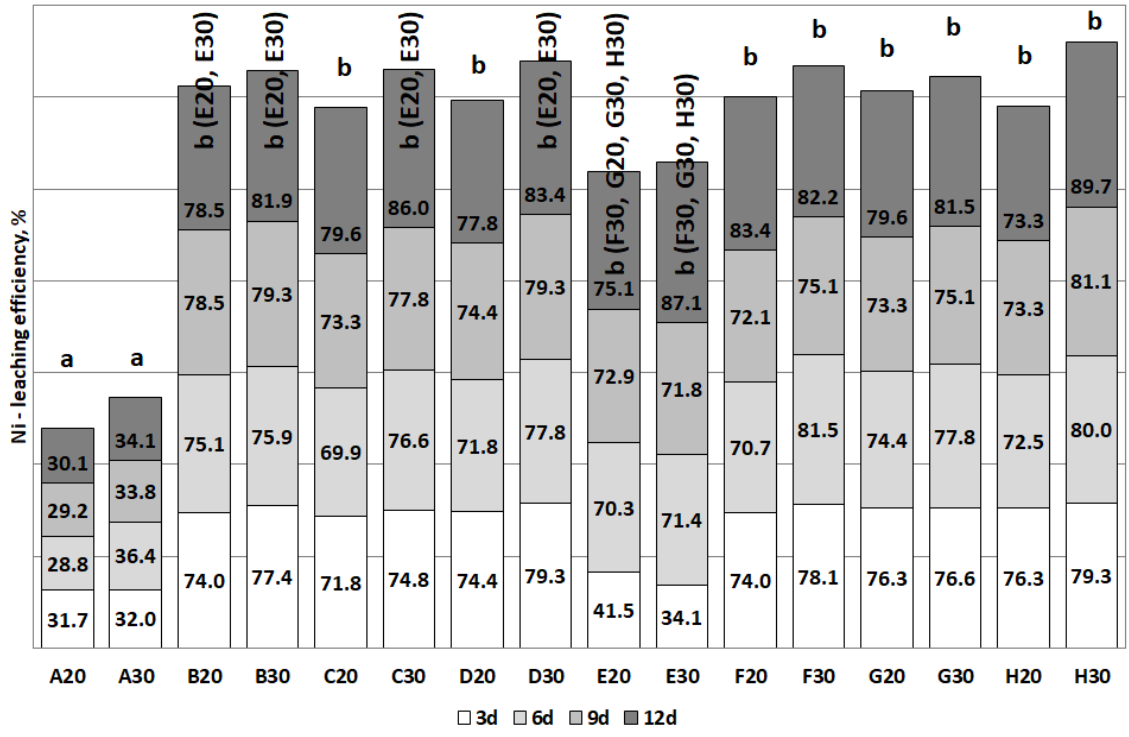

Figure 14. Bioleaching efficiency of Ni during the experiment (Statistical groups a and b).

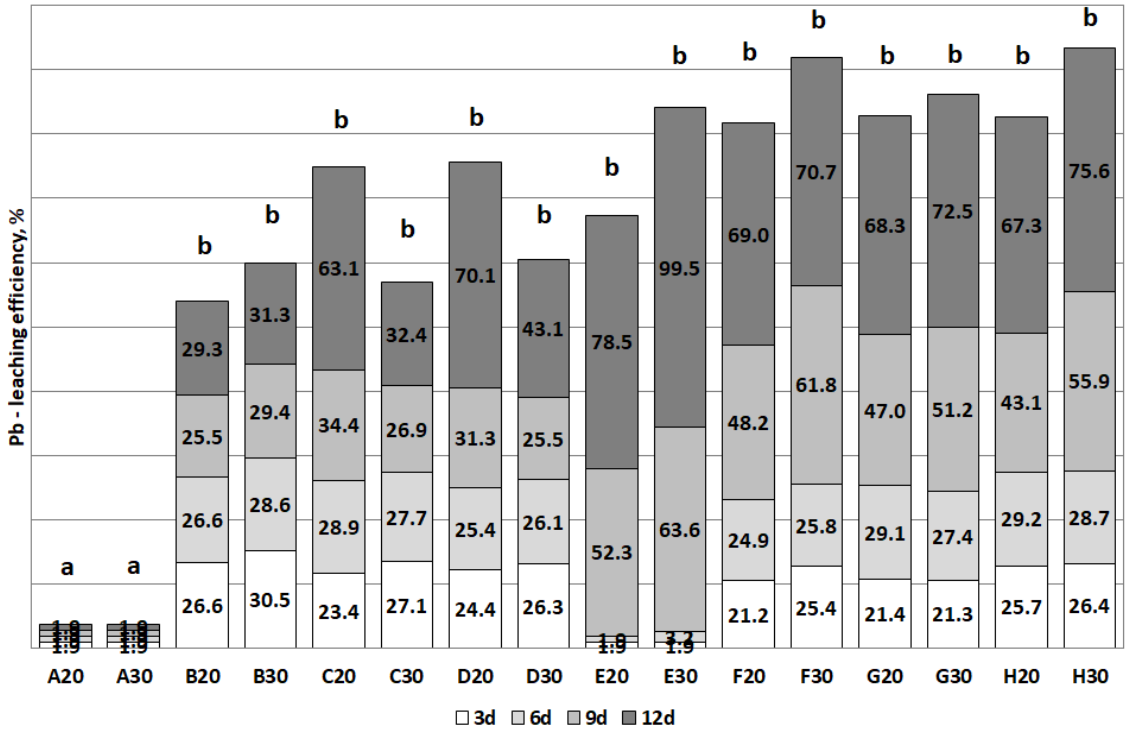

Figure 15. Bioleaching efficiency of Pb during the experiment (Statistical groups a and b).

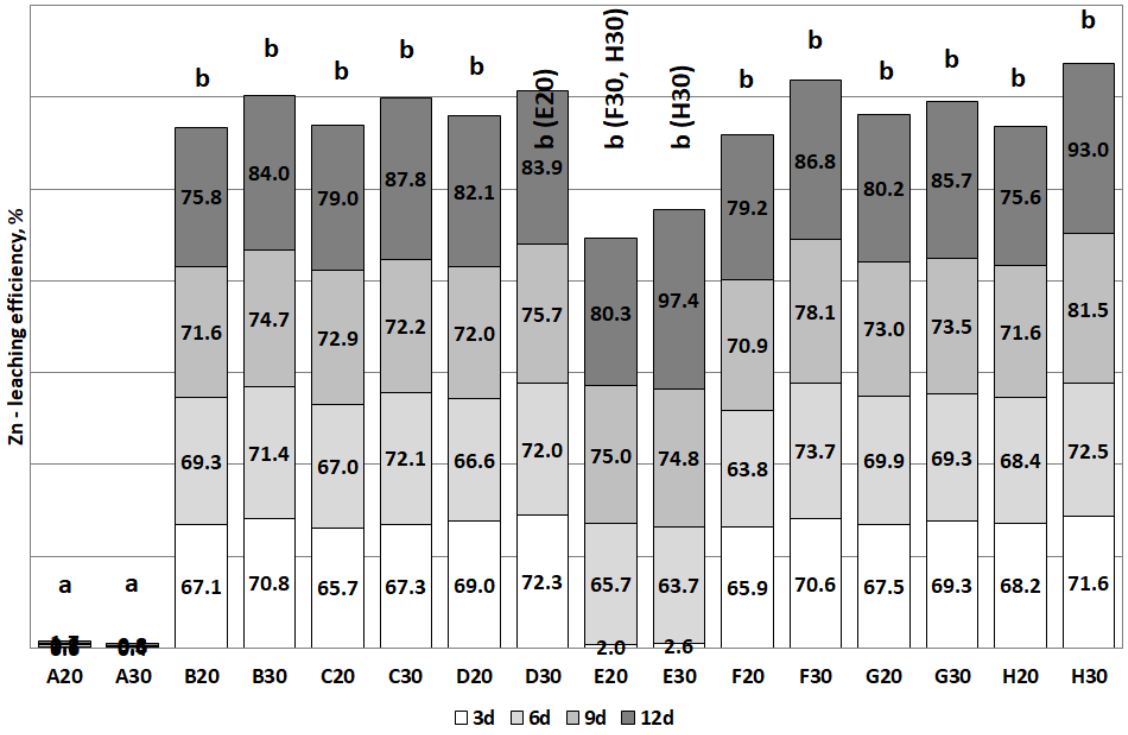

Figure 16. Bioleaching efficiency of Zn during the experiment (Statistical groups a and b).

The results for Al show that the release of the metal took place from the first days of the process; by the 3rd day, for most of the combinations, the efficiency was already between 20 and 30%. The highest Al bioleaching efficiency of 58.6% was obtained for the combination H20. Moreover, no significant differences were observed for the different incubation temperatures (Figure 6). The Ca bioleaching efficiency is shown in Figure 7. In the case of Ca, after 3 days of the process, an efficiency of over 70%

was already obtained for most of the combinations, except A and E. In this case, the temperature did not influence the efficiency of bioleaching. The highest value was obtained for combination E of 96.8% on day 12 of the experiment.

The course of Cd bioleaching for individual combinations was diverse. The highest efficiency was noted on the 12th day of the process for most treatments; however, there were significant differences between the combinations. The E30 trial achieved the highest efficiency, amounting to 68.4%; however, in the first days of the experiment (3 and 6), one of the lowest values was obtained. The results of the Cd bioleaching efficiency are presented in Figure 8. A similar distribution occurred for Cr, where also in the first days, the lowest efficiency values were obtained for combination E, 0.4% on the 3rd day and approximately 10% on the 6th day, while on the 12th, the value was the highest 91.9% (Figure 9).

A bioleaching efficiency above 50% for Cu could already be observed on day 3 of the process for most of the combinations, except for A and E (Figure 10). In the following days, no significant improvement was observed; only on the 12th day, the highest values of bioleaching efficiency were obtained, 80.6% for E30; a similar distribution was obtained for Al.

The highest efficiency of Fe bioleaching was obtained for the combination of E30 and H30, amounting to 95.6% and 93.7%, respectively. For the remaining samples, the distribution varied over time (Figure 11). Similarly, for Mg and for the combination E30, over 90% bioleaching efficiency was obtained. High bioleaching efficiency for all tests was achieved in the case of Mn, exceeding 80%. Moreover, the highest value of 99.5% among the tested combinations was obtained for E30 and H30 (99.3%); see Figure 13.

For the metals Ni, Pb, Zn, high bioleaching efficiency was recorded on the 3rd day of the experiment, except for A and E, for which higher activity was found on the 6th day. Moreover, for the discussed metals, the highest values were obtained for the combination of E and H (Figures 14–16).

Table 4 summarizes the highest bioleaching efficiencies depending on the incubation temperature (20 and 30 °C) for the control combinations (A–D) and the inoculated combinations (E–H). The efficiency of bioleaching at 20 °C was comparable for the A–D control combinations to the E–H inoculum combination. The effectiveness was in the range of 40% to 90%; most often, it was above 70%. Comparing the combinations A–D to E–H at 30 °C, it was found that most of the effectiveness (approximately 10%) was obtained for the samples inoculated with *A. thiooxidans*. In summary, it is concluded that the recovery of metals from sewage sludge from water treatment is highly effective. The obtained high metal recovery potential at the level of over 70% for all tested metals confirms the correctness of the proposed bioleaching process. Higher efficiencies (by approx. 10%) for the tested metals and used combinations were obtained for the temperature of 30 °C as compared to the temperature of 20 °C. The highest bioleaching efficiency for most metals was obtained for the inoculated combinations of E30 and H30. Combination E was without sulfur addition and without pH adjustment; the highest efficiency was obtained on day 12 of the process. For the combination with acidification of the sample (H30), the increase in yield was cyclical from the 3rd day of the process. It was found that acidification of the sample was crucial to accelerate the bioleaching process.

The leaching efficiency differed for several metals. According to Nareshkumar (2008), the bioleaching of Cr, Zn, Cu, Pb, and Cd from contaminated soil ranged from 11% to 99% with *A. thiooxidans* bacteria [46]. In the conducted study, the PCR results confirmed the species of the isolated bacteria strain belonging to *A. thiooxidans*. Xiang (2000) used *A. ferrooxidans* to deal with post-fermentation sludge and obtained the removal of Cr, Cu, Zn, Ni, and Pb which was between 16.2% and 91.5% [47]. The bioleaching of Al and Fe elements from coal fly ash using *Thiobacillus thiooxidans* (now reclassified as *A. thiooxidans*) was investigated. After 3 weeks of incubation, it resulted in extraction recovery yields of 25% Al and 22% Fe [33,48].

Table 4. Combinations with the highest leaching efficiencies.

Element	Incubation Temperature			
	20 °C	**30 °C**	**20 °C**	**30 °C**
	Combination (Efficiency, %)			
	Combinations A–D		**Combinations E–H**	
Al	C, D (>40)	B, C, D (>30)	H (>50)	E, H (>50)
Ca	B, C, D (>80)	C (>90)	E, G (>80)	E, H (>90)
Cd	D (>60)	D>20	G (>60)	E, F, G, H (>60)
Cr	D (>80)	C, D (>50)	G (>80)	E, H (>90)
Cu	B (>70)	B, C, D (>70)	E, F, G, H (>60)	E (>80)
Fe	D (>80)	D (>30)	F, G (>80)	E, H (>90)
Mg	B, C, D (>70)	C (>80)	E, F, G (>70)	E (>90)
Mn	D (>90)	B, C, D (>90)	E, F, G, H (>80)	E, G, H (>90)
Ni	B, C, D (>70)	B, C, D (>80)	F (>80)	E, F, G, H (>80)
Pb	D (>70)	D (>40)	E (>70)	E (>90)
Zn	D (>80)	B, C, D (>80)	E, G (>80)	E, H (>90)

According to Li (2012), the high efficiency of bioleaching may be caused by the activity of local bacteria [39]. The source from which the strains were isolated and the bioleaching was performed was the same; therefore, the used strain should be native with bacteria in the tested sample. Exogenous species often fail to contend with indigenous populations due to a lack of appropriate conditions and may inhibit the process. In addition to reducing costs, native bacteria are also more environmentally friendly, which offers the possibility of improving the condition of the environment. Such in situ action can promote bacterial metabolism and enhance the efficiency of bioleaching [49]. In addition, several studies have highlighted the effect of bioleaching time. However, in this study, we found that several types of metals can be absorbed by the sludge as the reaction time increases, which can reduce residues. Therefore, the reaction time is an important biological factor.

In this study, two incubation times were used, 20 and 30; after the obtained results, no significant differences were found. The effect of temperature on bioleaching has been extensively studied in the field of metal recovery from low-grade ores and mineral concentrates. In general, metal bioleaching is faster at higher temperatures, due to both an increase in the kinetic rate and a faster metabolism of S-oxidizing microorganisms (consequently, a sharp drop in pH) [29]. However, since most of the microorganisms used in bioleaching are mesophilic bacteria, excessively high temperatures can lead to inhibition. The highest leaching activity in the presence of mesophilic S-oxidizing strains is from approximately 30 to 35 °C [29,45,50–52]. Some other studies have confirmed these results also in the case of bioleaching of contaminated sludge [29,53]. However, in large-scale applications (e.g., fixed bed biological leaching), heat produced by exothermic reactions can build up, causing the temperature to rise. It seems that the greatest temperature fluctuations occur in the first days of treatment, which causes delays in S oxidation [29,54,55]. Bioleaching of metals from water treatment sludge with *A. thiooxidans* does not exhaust the potential of microorganisms capable of carrying out this process. Bacteria of the genus *A. ferrooxidans* are also commonly used [56]. In bioleaching studies, heterotrophic microorganisms such as *Aspergillus niger* and *Penicillium purpurogenum* and yeast (*Rhodotorula rubra*) are also used [57]. In order to increase the efficiency of bioleaching, the use of a differentiated biocenosis in two-stage bioleaching, such as *A. ferrooxidans–E. coli*, can be considered [58,59].

3.3. Mass Balance in Bioleaching

Figure 17 shows the obtained biomass and the TKN after a 12-day bioleaching experiment. The biomass at the beginning of the experiment was 8.9 g/L. In the case of combination A, on day 12, no increase in biomass was observed. From samples B to D20, a gradual increase in biomass to the value of 33 g/L can be seen. For combination E, a lower value of 18 g/L was noted, and then

a gradual increase to the value of 57 g/L for the H30 test. For all combinations, a higher value of biomass was found at 30 than 20 °C. The TKN concentration before the start of the experiment was 721 mg N/L. The highest concentration of TKN was obtained for the combination of E30 and H30. The TKN concentration was above 400 mg/L.

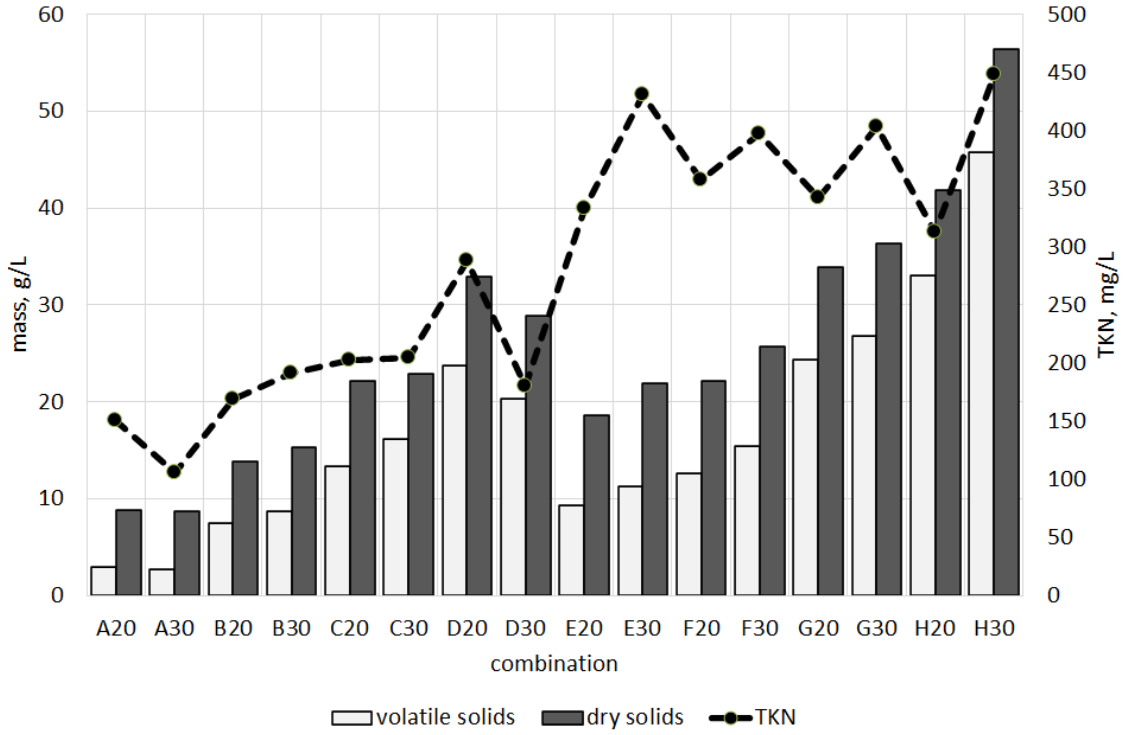

Figure 17. Mass balance during bioleaching for the tested combinations.

3.4. Statistical Analysis

Statistical analysis showed the occurrence of two groups of combinations between which the efficiency of bioleaching was significantly different. The first group (a) consists of combinations of A20 and A30. The second group (b) are combinations B–H. Importantly, within groups a and b, no statistically significant differences in the efficiency of bioleaching were noted. This means that in group (b), regardless of the factors used (acidification, inoculum, sulfur addition, temperature change), the metal leaching effect was comparable for all combinations. All exceptions are marked in the diagrams in parentheses with the different combinations indicated within the group, the effectiveness of which differed significantly from the effectiveness obtained for the combination in parentheses.

4. Conclusions

Bioleaching technology is environmentally friendly for metal recovery in sludge from water treatment. There is growing interest in heavy metal recovery technologies from various types of waste, but obtaining high efficiency requires the addition of reaction conditions and the optimization of individual parameters, ranging from bioleaching microflora, through process temperature, pH, and the amount of additives.

The presented study shows the high potential of metal recovery from sludge from water treatment. The effectiveness of the process for most metals was high (Ca 96.8%, Cr 92.6%, Cu 80.6%, Fe 95.6%, Mg 91%, Mn 99.5%, Ni 89.7%, Pb 99.5%, Zn 93%); only lower values were obtained for Al 58.6% and Cd 68.4%. However, in order to increase the efficiency for these metals, additional methods of disintegration of the substrate before the process can be introduced; this will be the subject of further research. The highest bioleaching efficiency for most metals was obtained for the combination of E30

and H30. The E combination was without the addition of sulfur and no pH adjustment, which was noticeable especially in the first days of the process (day 3) due to the low efficiency of bioleaching, but at the end, on the 12th day of the experiment, high efficiency was noted. For the H-combination with all additives, the high efficiency of the process was visible from the very beginning; as early as on day 3, the efficiency was approximately 70% for the tested metals. Based on the results obtained, bioleaching technology is expected to see significant breakthroughs in the not-so-distant future.

Author Contributions: Conceptualization, T.K. and M.W.; methodology, T.K.; software, T.K. and M.W.; validation, T.K.; formal analysis, M.W.; investigation, M.W.; resources, T.K.; data curation, T.K.; writing—original draft preparation, M.W.; writing—review and editing, M.W.; visualization, T.K.; supervision, M.W.; project administration, M.W.; funding acquisition, T.K. and M.W. All authors have read and agreed to the published version of the manuscript.

Funding: The study was carried out in the framework of the statutory funds for research, financed by the Ministry of Science and Higher Education.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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