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Water purification sludge as a substrate in metal bioleaching

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ABSTRACT

The presented work analyses the efficiency, suitability and application prospects of bioleaching (by *Acidithiobacillus thiooxidans*) of metals from low contaminated waste – post coagulation iron sludge from groundwater purification. The test results showed a high bioleaching efficiency of over 90% for iron, but also for aluminium, manganese, calcium, magnesium and chromium. Lead, nickel and cadmium were in the sludge, although at low concentrations, were solubilised to 70%–80%. Process time was an important factor for the bioleaching effect. It was necessary to run the bioleaching process as a batch culture for at least 6 d. The efficiency of bioleaching was found to be independent of temperature. Both cultivation at 10°C and 20°C were suitable for the experiment and the achieved metal solubilisation efficiency was very comparable. Acidification of the sample was a key factor in the efficiency of the bioprocess. The inoculation of the studied sludge and the addition of elemental sulfur as an energy substrate were not significant factors for the solubilization of metals. The acidified sludge from groundwater purification was classified as a semi-finished iron (waste) coagulant and a good substrate for the growth of bioleaching microorganisms.

Keywords: Regeneration of iron post-coagulation sludge; Bioleaching; *Acidithiobacillus thiooxidans*; Heavy metals; Light metals; Biomass growth

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1. Introduction

Sludge produced in a water purification plant (WPP) is a liquid waste generally generated by the coagulation and filtration process. The quality of the sludge produced depends largely on the type of water used to treat, that is, surface or groundwater. Sludge from surface water treatment contains large amounts of pollutants, including hazardous organic substances, heavy metals and even pathogens, which pose a significant threat to environment and human health [1–3]. The composition and properties of sludge are also largely determined by the reagents used in water purification such as iron or aluminium salts or organic flocculants. High levels of iron and aluminium are toxic to living organisms [4,5]. On the other hand, the content and properties of iron, and especially aluminum, are the elements that make post-coagulation sludge worthy of valorization and recycling.

A relatively simple way to manage sludge from water purification is to apply it to land for the growth of agricultural products. This is due to the presence of micro and macro nutrients and organic matter [6,7]. There is also a possibility of direct use of post-coagulation sludge in the production of bricks, roof tiles [8], as materials in road construction [9], geotechnical work [10] or processing into biochar [11]. Water purification sludge can be used as a waste coagulant to remove specific pollutants such as biochemical and chemical oxygen demand or suspensions from municipal [12] or industrial and agricultural wastewater [13]. It is also reasonable to use post-coagulation sludge in wastewater treatment technology as a low-tech and low-cost absorber [14] for sorption of phosphorus, heavy metals [15] or for nitrate removal [16]. However, the direct use of sewage sludge is not straightforward. It should be noted that the addition of post-coagulation sludge in different technologies determines the properties of the resulting products or wastes.

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The presence of a significant amount of various types of hazardous substances in post-coagulated sludge encourages the use of technologies that allow the removal of the pollutants and then recover them as a high quality coagulant or absorber [17]. Examples of such technologies include the use of ultrasound to degrade organic pollutants [18], the synthesis of hydrochar from post-coagulation sludge for phosphorus adsorption [19] or the recovery of aluminum using ammonium sulfate roasting process [20].

A well-known method that allows the regeneration of postcoagulant sludge to coagulant is the use of membrane processes [21]. Another, simpler technology that enables recovery of the coagulant (aluminum or iron) is acidification [22]. Acidification can be carried out by chemical leaching with sulfuric, hydrochloric and nitric acids [23]. The main disadvantage of coagulant recovery by acidification is the release of organic matter and many other metals from sludge [24]. Since acidification is required for the regeneration of sludge after coagulation, the use of bioleaching can replace chemical technology. Bioleaching is a natural process but is also classified as a biohydrometallurgical process. The fundamental importance of industrial bioleaching has been developed as a method of recovering metals from ores with low leachable element content. The process involves the growth of microorganisms, usually at very low pH. *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans* are the most commonly used bacteria in bioleaching. To produce energy, *A. thiooxidans* oxidizes sulfur compounds to sulfuric acid. *A. ferrooxidans* oxidizes Fe^{+2} to Fe^{+3} using both iron and sulfur compounds to generate energy. Finally, lowering the pH even to pH 1.0–3.0 and the formation of sulfuric acid leads to the solubilization of metals, generally without a negative impact on the metabolism and growth of these bacteria. However, from the technical and technological side, however, bioleaching is not the simplest process. High efficiency of bioleaching/solubilization of metals requires appropriate process conditions, including nutrient demand, an aerobic reaction environment and the optimum temperature for microorganism development of 30°C [25,26].

In previous studies [27], sludge from groundwater purification was bioleached using *A. thiooxidans* at 30°C. The high iron content and low heavy metal content of the sludge allowed the regeneration of the post-coagulation sludge for the recovery of the coagulant (iron salts) and the production of biomass of bioleaching bacteria. In the present study, the leaching/bioleaching process was carried out at 10°C and 20°C. Reducing the process temperature was considered crucial for rationalising the process, that is, reducing the energy consumption of the process. The tested water purification sludge was collected from a buffer tank with a temperature range of 10°C–20°C. The obtained results may be useful for possibly changing the function of the buffer tank into a biological reactor. The use of *A. thiooxidans* bacteria requires aeration of the reactor, which cannot be omitted and constitutes an additional cost of the tested technology. The second main objective of the research was to test whether the sludge could be a medium for the growth of biomass of bioleaching bacteria.

Table 1
Physical and chemical characteristics of the tested water processing sludge

Parameter	Mean, standard deviation
pH	7.6 ± 0.1
Dry solids (DS), g/L	2.5 ± 0.2
Volatile solids (VS), g/L	1.3 ± 0.1
VS in DS, % DS	51.8 ± 0.3
Total Kjeldahl nitrogen, mg·N/L	253.1 ± 0.6
Ammonium nitrogen, mg·N–NH^{4+}/L	26.0 ± 0.8
Total Kjeldahl nitrogen, dissolved form, mg·N/L	10.6 ± 0.6
Ammonium nitrogen, dissolved form, mg·N–NH^{4+}/L	8.7 ± 0.3
Alkalinity, mg·$CaCO_3$/L	310.0
Capillary suction time, s	47 ± 7
Carbon content, % DS	26.30 ± 1.2
Hydrogen content, % DS	5.12 ± 0.33
Nitrogen content, % DS	5.1 ± 0.11
Sulfur content, % DS	0.91 ± 0.05

Table 2
Concentrations of tested metals in the collected sludge

Element	Concentration (µg/L)	Metal content (mg/kg DS)	Metal content (% DS)
Aluminum (Al)	3,041.7 ± 73.6	1,216.7	0.1217
Calcium (Ca)	95,358.94 ± 626.0	38,143.6	3.8144
Cadmium (Cd)	30.50 ± 1.6	12.2	0.0012
Chrome (Cr)	965.79 ± 7.9	386.3	0.0386
Copper (Cu)	162.18 ± 1.6	64.9	0.0065
Iron (Fe)	321,117.48 ± 2,659.5	128,447.0	12.8457
Magnesium (Mg)	11,064.64 ± 93.3	4,425.9	0.4426
Manganese (Mn)	1,187.55 ± 3.2	475.0	0.0475
Nickel (Ni)	70.49 ± 3.0	28.2	0.0028
Lead (Pb)	270.05 ± 11.1	108.0	0.0108
Zinc (Zn)	32,51.52 ± 16.6	1,300.6	0.1301

2. Research methodology

2.1. Substrate

A mixture of post-coagulation sludge and backwash water from the groundwater purification process was tested (Tables 1 and 2). The technological system of the WPP consists of bioreactors for denitrification, reactors for aeration, coagulation and settling tanks and finally double-layer filters (mineral filling – activated carbon). The water is disinfected by ozonation. Water is taken from aquifers lying at a depth of 40–70 m. The capacity of the water purification plant is 750 m^3/d.

The use of denitrification reactors results from the contamination of groundwater with nitrogen compounds as a result of improper sewage and agricultural land management in the last century. $FeCl_3$ is used for coagulation.

Sludge and backwash water from water purification processes are collected in a buffer tank and then dewatered using chamber works. The substrate collected from the buffer tank was defined as water processing sludge (WPS). No measuring system was installed in the facilities of the water purification plant to monitor the volume of post-coagulation sludge and backwash water. According to the technological personnel, the volume fraction of backwash water in the mixture with sludge was considered insignificant.

2.2. Inoculum

A bacterial suspension of *A. thiooxidans* was used to inoculate WPS samples. The culture of *A. thiooxidans* on the WPS medium was performed according to the according to the methodology proposed by Li et al. [28]. The 9 K medium with the composition: 3 g/L $(NH_4)_2SO_4$, 0.5 g/L $MgSO_4·7H_2O$, 0.5 g/L $K_2HPO_4·3H_2O$, 0.1 g/L KCl, 0.01 g/L $Ca(NO_3)_2$, 1 L of distilled water and 10 g/L of elemental sulfur (S^0) was used for bacterial culture. The culture was performed at two temperatures: 10°C and 20°C. The optimal temperature for the growth of acidic bacteria *A. thiooxidans* is 30°C [28,29,31]. Lowering the temperature of the culture was consistent with the objective of the research, which was to determine the bioleaching efficiency and biomass growth at temperatures lower than optimal. After 5 cycles of 7-d incubation, the bacterial suspension was subjected to mass separation. Centrifugation (3,000 rcf, 2 min) was used to separate the bacterial biomass from the post-culture solution. The liquid was then decanted and the concentrated biomass was dissolved in PBS (phosphate buffered saline) medium. The obtained bacterial suspension (inoculum) was characterized by a dry solids (DS) of 34.2 g/L (10°C) and 37.5 g/L (20°C). The volatile solids of inoculum was 73.8% and 78.5% DS, respectively.

2.3. Research methodologies

Experiments were performed as 12-d batch cultures. Reactors with an active volume of 0.3 L were used, stirred and aerated by rotation (180 rpm/min.). The process temperature was set at 10°C and 20°C. Seven test combinations were used, including the addition of bacterial inoculum and the conditions that determine the growth of *A. thiooxidans*: pH 2.0 correction, culture temperature 10°C and 20°C, addition of 10 g/L elemental sulfur (optimal dose for the growth of *A. thiooxidans*). No sulfur addition was used in combinations A, B, D and E. This energy substrate as well as micro and macro elements have been replaced by ingredients contained in WPS. The last combination was a batch culture in anaerobic conditions – fermentation (Table 3).

Combinations A, B, C were control combinations, with distilled water added instead of the inoculum. Taking into account the dry and volatile solids of the inoculum and the volume ratio of substrates (Table 3), 0.75 and 0.88 g biomass of bioleaching bacteria were dosed into the tested samples (D, E, F) incubated at 10°C and 20°C, respectively.

2.4. Physicochemical analyses

The assessment of bioleaching efficiency was based on the monitoring of pH, redox potential and concentrations of selected elements: Al, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Zn.

Table 3
Leaching/bioleaching research combinations

Research combination		Volume ratio of substrates	pH correction	Sulfur addition, g/L	Incubation temperature, °C
A	A10	WPS/Water	–	–	10
	A20	9:(9/1)1			20
B	B10	WPS/Water	2.0	–	10
	B20	(9/1)			20
C	C10	WPS/Water	2.0	10	10
	C20	(9/1)			20
D	D10	WPS/*A. thiooxidans*	–	–	10
	D20	(9/1)			20
E	E10	WPS/*A. thiooxidans*	2.0	–	10
	E20	(9/1)			20
F	F10	WPS/*A. thiooxidans*	2.0	10	10
	F20	(9/1)			20
G	G10	WPS	–	–	10
	G20	(Digestion)	–	–	20

The analyses were carried out on the 6th and 12th day of the process, the mass analysis of the obtained biomass and its susceptibility to separation (capillary suction time, CST) were performed after the end of the experiment.

Physical and chemical analyses were carried out using appropriate standards:

- The dry solids (DS) – PN-EN 12880:2004; volatile solids (VS) PN-EN 12879:2004; total Kjeldahl nitrogen (TKN) – PN-EN 16169:2012; Ammonium nitrate (N–NH_4) PN-EN 14671:2007; alkalinity – PN-EN ISO 9963-1.; capillary suction time – PN-EN 14701-1:2007,
- The reduction-oxidation potential was determined by the electrochemical method, the pH was determined by the potentiometric method,
- Metals were determined in accordance with the PN-EN ISO 11885:209 standard using an atomic emission spectrometry apparatus (ICP-OES; Thermo Apparatus, USA),
- The elemental content of carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) was performed using LECO TruSpec multi analyzer.

Concentrations of ammonium nitrogen, Kjeldahl nitrogen and heavy and light metals were determined in samples of sludge liquids as the dissolved fraction. For this purpose, the samples were centrifuged for 15 min at an overload of 12,100 rcf. The decanted supernatant was filtered through a cellulose acetate membrane (0.45 µm).

The metal leaching/bioleaching results are presented in terms of leaching efficiency (LE) as:

$$\text{LE} = \frac{C_{\text{WPS}} - C_{\text{DF}}}{C_{\text{WPS}}} \times 100\%$$

where C_{WPS} – metal concentration in WPS, µg/L; C_{DF} – metal concentration in dissolved form (examined on the 6th and 12th day of the process), µg/L.

2.5. Statistical analysis

Analysis of variance (ANOVA) was used to test the significance of differences in bioleaching efficiency. The null hypothesis was that there were no differences in bioleaching efficiency (LE) between the samples tested, despite differences in formulation (combinations A10-G20). For statistically significant data $p < 0.05$, Tukey's test was used (post-hoc analysis). The results of the analysis allowed to determine 4 groups (a, b, c, d), between which there was a statistically significant difference. Samples (combinations) assigned to the appropriate group are marked with the letters a, b, c, d in the LE graphs. Samples (combinations) assigned to the same group showed no statistically significant differences in leeching/bioleaching efficiency (LE).

3. Research results

Changes in pH and oxidation–reduction potential are the basic indicators in bioleaching monitoring. Possibly low pH values and possibly high redox potential are considered effective conditions for the bioleaching process.

Due to the pH of the reaction environment, the correct conditions for biomass development of bioleaching bacteria were in the samples of combinations B, C, E, F (irrespective of the incubation temperature), (Fig. 1). In all listed combinations, the pH of the samples was close to pH 1.0. On the contrary, a pH of 6–8 in the Combination A samples may have stimulated the growth of other types of microorganisms. In the samples of combination D (10°C, 20°C), that is, unacidified samples with the addition of inoculum, the pH of the samples was close to pH 2.0 only on the 12th day of the process. This may be due the slow growth of bioleaching bacteria and their metabolism, which successively acidifies the environment.

Similar trends were noted during the measurements of the oxidation–reduction potential (Fig. 2). Samples of combinations B, C, E, F are characterized by a stable redox value of nearly 500 mV. Such a high potential confirmed the intense oxidation reactions. A lower redox value, of the order of 300 mV, was characteristic of untreated samples (combination A). Intermediate redox values, that is, about 400 mV, were recorded for the D combination samples.

The statistical analysis provided very important conclusions regarding the bioleaching efficiency of the elements. The results of statistical research showed the existence of

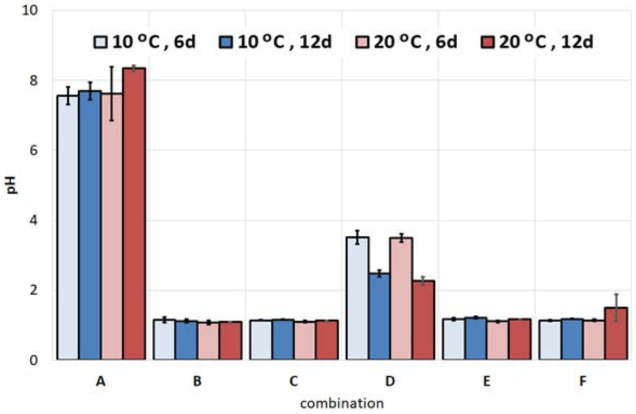

Fig. 1. Changes in pH of prepared water processing sludge samples.

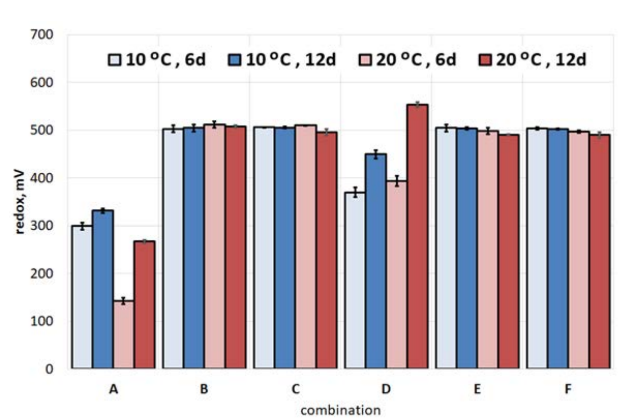

Fig. 2. Changes in redox potential of prepared water processing sludge samples.

three groups of tested combinations. There were significant differences in the leaching efficiency of the elements between these groups. What is very important, these groups depended on the method of preparation, and not on the incubation temperature or the process day.

The first group (a) consists of samples of combination A, which was found to be the least effective. These were samples only incubated at 10°C and 20°C, no additives were used in this case. The leaching efficiency of Al, Cr, Fe, Mn, Pb and Zn generally did not exceed 10%. Monitoring of Ca, Cd, Cu, Mg and Ni concentrations showed that the solubilization of these elements ranged from 20%–50% (Figs. 3–13).

The second group (b) consisted of samples of combinations B, C, E and F. The common denominator of these samples was their acidification to pH 2.0. Differences in the method of preparation resulted from the addition of inoculum and sulfur. For these combinations, the leaching efficiency ranged from 70% to 90%. The second group also included samples from combination D, that is, Ca, Mg, Mn, whose solubilization was close to 80% (Figs. 3–13).

The third group (c) included most of the combination D samples, that is, samples only inoculated with *A. thiooxidans*. In this group the leaching efficiency did not exceed 20% for Al, Cr, Fe, 40% for Pb and Cd and 70% for Ni and Zn (Figs. 3–13).

A separate group (d) consisted of G10 and G20 fermented samples. A better metal solubilisation effect was observed for the G10 combination, although the statistical analysis

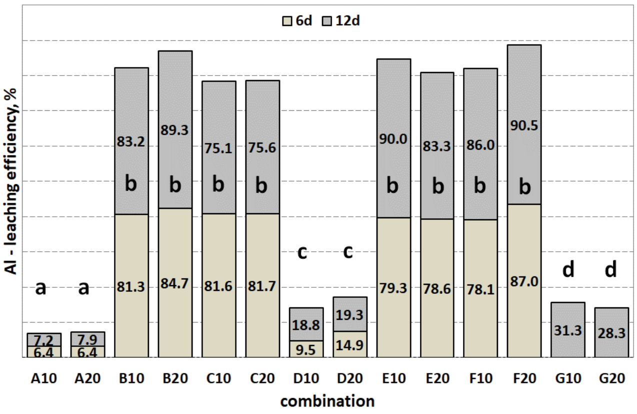

Fig. 3. Concentrations of solubilized Al in the supernatant of the prepared water processing sludge.

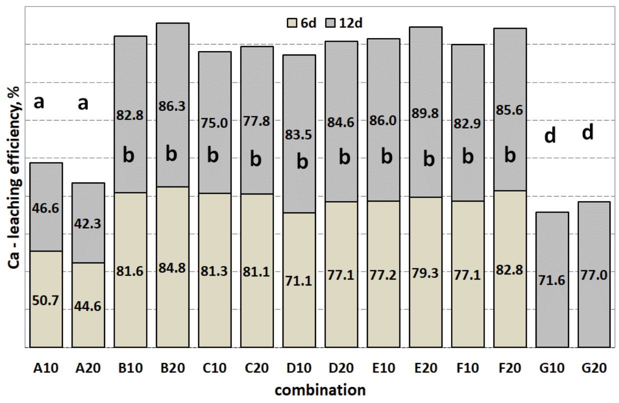

Fig. 4. Concentrations of solubilized Ca in the supernatant of the prepared water processing sludge.

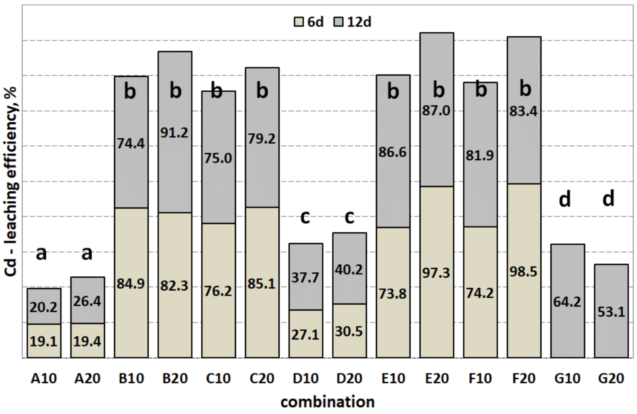

Fig. 5. Concentrations of solubilized Cd in the supernatant of the prepared water processing sludge.

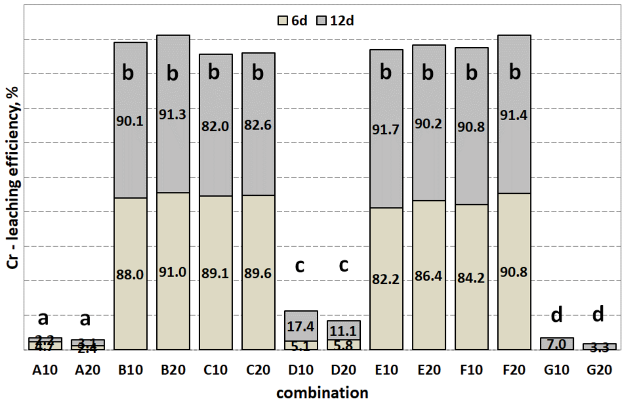

Fig. 6. Concentrations of solubilized Cr in the supernatant of the prepared water processing sludge.

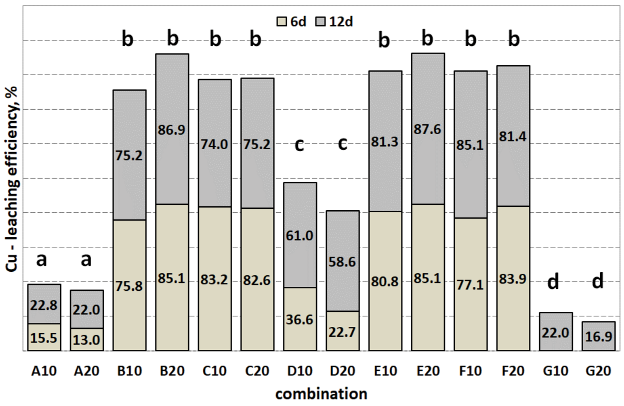

Fig. 7. Concentrations of solubilized Cu in the supernatant of the prepared water processing sludge.

showed that there were no significant differences between these combinations. The lowest efficiency was obtained for Cr, Zn (<10%), and intermediate for Al, Cu, Fe (<40%). For a series of elements Ni, Pb, Cd, Ca, Mn, Mg, the maximum solubilization efficiency was obtained, amounting to 54%, 58%, 64%, 77%, 82% and 84%, respectively (Figs. 3–13).

Based on the most advantageous combinations (B, C, E, F) the efficiency series of leached heavy metals were analysed (Table 4). However, no clear similarities between individual combinations were found. The general pattern was that the series of leaching efficiencies started with lead and nickel and cadmium. The concentration of Pb, Ni and Cd

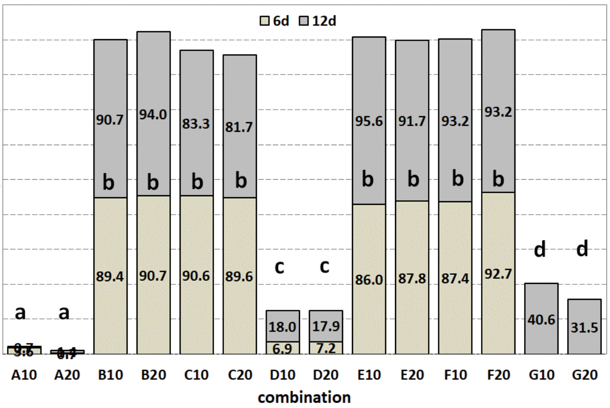

Fig. 8. Concentrations of solubilized Fe in the supernatant of the prepared water processing sludge.

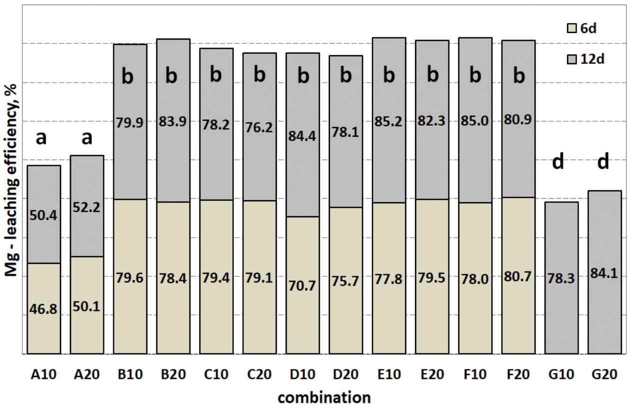

Fig. 9. Concentrations of solubilized Mg in the supernatant of the prepared water processing sludge.

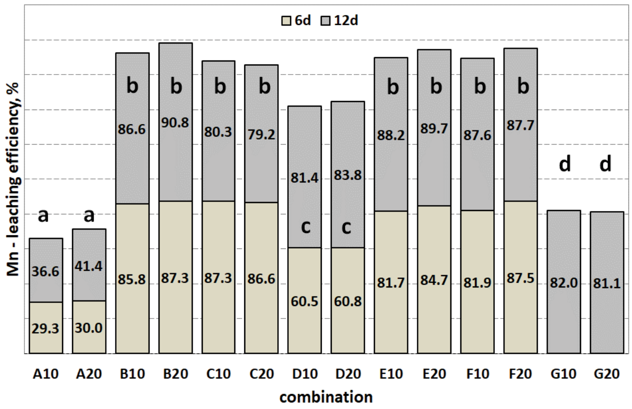

Fig. 10. Concentrations of solubilized Mn in the supernatant of the prepared water processing sludge.

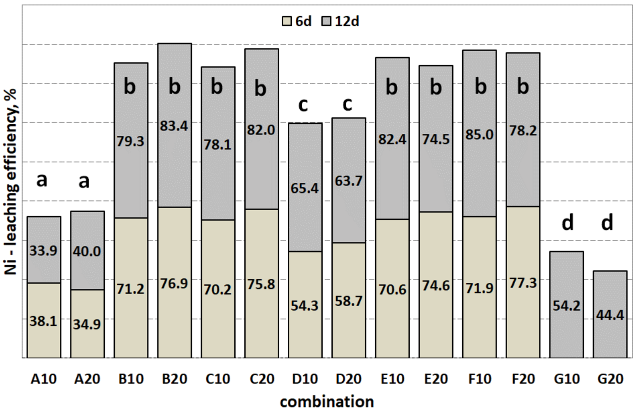

Fig. 11. Concentrations of solubilized Ni in the supernatant of the prepared water processing sludge.

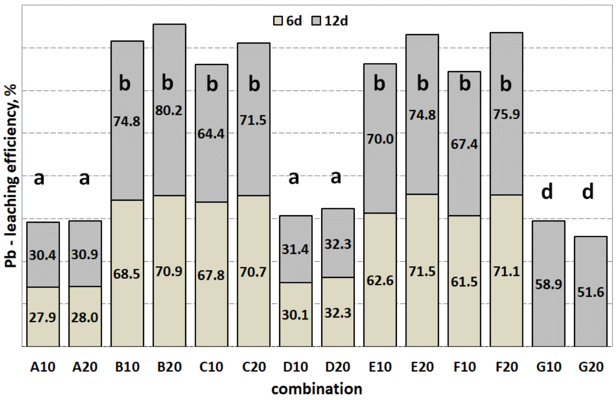

Fig. 12. Concentrations of solubilized Pb in the supernatant of the prepared water processing sludge.

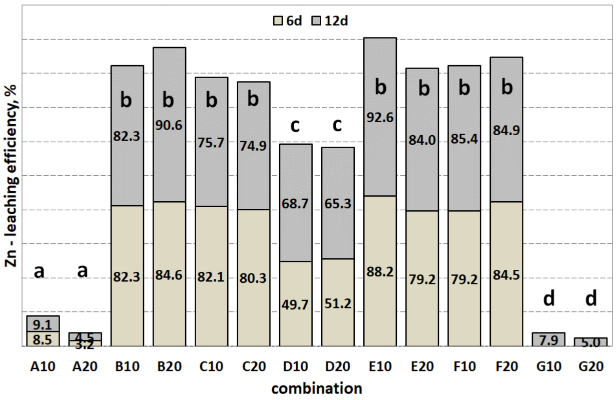

Fig. 13. Concentrations of solubilized Zn in the supernatant of the prepared water processing sludge.

in dissolved form after 12-d culture was on average 72%, 69% and 69%. The best leaching metals were Cr and Zn. Chromium was present in dissolved form in about 90% of the content in the sludge, and zinc in about 85%.

Comparing the LE series of light metals, only general regularity was also noted (Table 5). Most often, the first in the series was magnesium (average 81%), while the highest leaching efficiency was characteristic of manganese (average 86%), and especially iron (over 90%).

To illustrate the concentrations of leached metals in the tested samples, they were compared to the limit values contained in two national (Polish) legal acts (Table 6). These were the regulations on the conditions for discharging wastewater into sewage systems [29] and the regulation on substances particularly harmful to the aquatic environment and the conditions to be met when discharging wastewater into waters or into the ground [30].

The concentrations of heavy metals in the tested combinations did not exceed the listed limit values. This means that these liquids can be discharged without pre-treatment into the sewerage system or even into a watercourse, in accordance with national legislation. The exception was the content of chromium (0.88 mg/L) in samples B20, F10, F20. In this case, the only options are of entering the sewerage system for further treatment.

It was found that the concentrations of leached heavy metals did not pose a significant threat to the environment. This was due to the fact that the tested sludge was produced in the process of high-quality groundwater purification. On the other hand, water purification with the use of iron coagulants resulted in very high concentrations of iron in the tested samples (262–307 mg/L – combinations B, C,

Table 4
Efficiency of solubilization of heavy metals on the 12th process day in selected combinations

	Element	Ni	Cd	Pb	Cu	Zn	Cr
B10	LE, %	73.9%	74.4	74.8	75.2	82.3	90.1%
	mg/L	0.05	0.02	0.20	0.12	2.68	0.87
	Element	Pb	Ni	Cu	Zn	Cd	Cr
B20	LE, %	80.2	83.4	86.9	90.6	91.2	91.3
	mg/L	0.22	0.06	0.14	2.95	0.03	0.88
	Element	Pb	Cu	Ni	Cd	Zn	Cr
C10	LE, %	64.4	74.0	74.2	75.0	75.7	82.0
	mg/L	0.17	0.12	0.05	0.02	2.46	0.79
	Element	Pb	Zn	Cu	Cd	Ni	Cr
C20	LE, %	71.5	74.9	75.2	79.2	82.0	82.6
	mg/L	0.19	2.43	0.12	0.02	0.06	0.80
	Element	Pb	Cu	Ni	Cd	Cr	Zn
E10	LE, %	70.0	81.3	82.4	86.6	91.7	92.6
	mg/L	0.19	0.13	0.06	0,03	0.89	3.01
	Element	Ni	Pb	Zn	Cd	Cu	Cr
E20	LE, %	74.5	74.8	84.0	87.0	87.6	90.2
	mg/L	0.05	0.20	2.73	0.03	0.14	0.87
	Element	Pb	Cd	Ni	Cu	Zn	Cr
F10	LE, %	67.4	81.9	85.0	85.1	85.4	90.8
	mg/L	0.18	0.02	0.06	0.14	2.78	0.88
	Element	Pb	Ni	Cu	Cd	Zn	Cr
F20	LE, %	75.9	78.2	81.4	83.4	84.9	91.4
	mg/L	0.21	0.06	0.13	0.03	2.76	0.88

Table 5
Efficiency of solubilization of light metals on the 12th process day in selected combinations

	Element	Mg	Ca	Al	Mn	Fe
B10	LE, %	79.9	82.8	83.2	86.6	90.7
	mg/L	8.85	78.97	2.53	1.03	291.35
	Element	Mg	Ca	Al	Mn	Fe
B20	LE, %	83.9	86.3	89.3	90.8	94.0
	mg/L	9.28	82.29	2.72	1.08	301.85
	Element	Ca	Al	Mg	Mn	Fe
C10	LE, %	75.0	75.1	78.2	80.3	83.3
	mg/L	71.55	2.28	8.65	0.95	267.56
	Element	Al	Mg	Ca	Mn	Fe
C20	LE, %	75.6	76.2	77.8	79.2	81.7
	mg/L	2.30	8.43	74.17	0.94	262.50
	Element	Mg	Ca	Mn	Al	Fe
E10	LE, %	85.2	86.0	88.2	90.0	95.6
	mg/L	9.42	82.04	1.05	2.74	307.13
	Element	Mg	Al	Mn	Ca	Fe
E20	LE, %	82.3	83.3	89.7	89.8	91.7
	mg/L	9.10	2.53	1.07	85.62	294.61
	Element	Ca	Mg	Al	Mn	Fe
F10	LE, %	82.9	85.0	86.0	87.6	93.2
	mg/L	79.07	9.41	2.62	1.04	299.35
	Element	Mg	Ca	Mn	Al	Fe
F20	LE, %	80.9	85.6	87.7	90.5	93.2
	mg/L	8.95	81.66	1.04	2.75	299.19

Table 6
Permissible concentrations of heavy metals according to selected legal acts

Element	Discharging wastewater into sewage systems [29]	Discharging wastewater into waters or into the ground [30]
	Maximum permitted concentrations, mg/L	
Cd	Max. 0.4	Max. 0.4
Cr	1.0	0.5
Cu	1.0	0.5
Ni	1.0	0.5
Pb	1.0	0.5
Zn	5.0	2.0
Al	*	3.0
Ca	*	*
Fe	*	10.0
Mg	*	*
Mn	*	*

* - Limited by the company responsible for the sewer network and sewage treatment plant.

E, F). However, in this article, iron is not considered as an pollution but as a coagulation cation. In combination with chemically or biologically oxidized sulfur (dosed into samples), a waste coagulant (iron sulphate) of potentially high quality can be obtained.

At the end of the process (12 d), the concentrations of dry matter and dry organic matter in the samples were determined (Fig. 14). The determined DS and VS values allow the assessment of the influence of culture conditions on the growth of microorganisms. The lowest dry matter content was recorded in samples prepared with combination A (max. 2.6 g/L) and in combination D (max. 6.4 g/L). The dry weight and organic dry weight of the fermented samples (G) was also negligible (≈3.0 g/L), but also independent of the incubation temperature.

A high dry solids content was noted for combinations B, C, E and F and was almost 20 g/L (Fig. 14). This means that the conditions of sample preparation in combinations B, C, E and F were favorable for biomass growth. This is confirmed by the content of volatile solids, which was even 80%–90% of the dry solids of the samples. What is very important, the increase in biomass occurred in combinations B and E, in which no sulfur was added. It can therefore be stated that the WPS is a good microbiological medium for the growth of microorganisms. The increase which occurred at both higher (20°C) and lower (10°C) incubation temperatures, is also important. Comparing the DS and VS values, it is possible to conclude that acidifying the samples was the key factor in microbial development. The addition of inoculum and the addition of sulfur as an energy substrate were of minor importance.

The measurement of the capillary suction time (as in the case of sewage sludge) was important the susceptibility of the prepared sludge to separation of the solid phase from the solution (Table 7). The efficiency of solid phase separation will be important for the recycling of the obtained products, that is, biomass and concentrated sludge liquids. CST values were the most favorable for samples incubated at 20°C (max. 168 s, E20). Also, the sludge samples subjected to fermentation (G10, G20) did not show a significant increase in CSK (max. 165 s, G20). On the other hand, the samples incubated at 10°C were characterized by a high increase in CST, which is tantamount to deterioration of efficiency in solid phase separation processes. A multiple increase in CSK was demonstrated for the samples of the combination C10 (824 s) and F10 (983 s). This means that the separation of biomass, for example, by filtration, may be an inaccurate and, above all, time-consuming process.

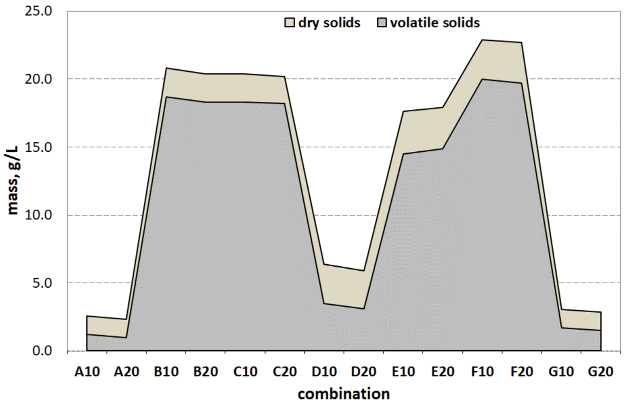

Fig. 14. Dry matter and dry organic matter of treated water processing sludge.

4. Discussion and application prospects

In the presented work, the efficiency of leaching/bioleaching metals from WPS was even 70% to 90%. However, this is not such a significant result. Many authors using the bioleaching technology have obtained comparable efficiency of metal extraction from various types of liquid and solid waste. Akinci and Guven [31] obtained about 80% efficiency of solubilization of copper and 90% of zinc from contaminated sediment. In studies by Wen et al. [32] the removal efficiencies of Cu, and Zn in sewage sludge was 96.2% and 96.5%, respectively. Potysz et al. showed that [33] up to 79% Cu, 76% Zn and 45% Fe could be extracted from crystalline slag. Naseri et al. [34] using *A. thiooxidans* for recovery of metals from spent coin cells obtained 99%, 60% and 20% recovery rates of Li, Co and Mn, respectively. Priya and Hait [35] applied a hybrid technology based on a combination of bioleaching and the chelating effect of citric acid for the extraction of metals from a high-grade waste printed circuit board. The maximum leaching efficiency obtained was 94% Cu, 92% Zn, 64% Pb and 81% Ni. Chen and Lin [36] state that bioleaching can be qualified as an efficient and cost-effective alternative to physicochemical soil treatment technologies. The authors concluded that the maximum efficiency of solubilization of most metals, amounting to over 80%.

The first common part of experiments on bioleaching metals from various wastes is usually the careful selection of process conditions, including particle size, pH, types of bioleaching bacteria, as well as temperature and aeration conditions. The second common part is the bioleaching process itself, implemented for many purposes, that is, metal recovery, environmental remediation, and also, as in the presented studies, direct production of a useful product from waste, that is, waste coagulant and biomass.

Table 7
Results of the capillary suction time test as a measure of the susceptibility to solid phase separation

Incubation temp.	Capillary suction time, s						
	A	B	C	D	E	F	G
10°C	76 ± 11	506 ± 39	824 ± 103	166 ± 32	744 ± 180	983 ± 155	78 ± 4
20°C	50 ± 3	90 ± 13	106 ± 29	30 ± 6	168 ± 38	106 ± 9	165 ± 29

The bioleaching process played a large role, especially in the twentieth century, in the recovery of valuable metals from various sulphide minerals or low grade ores. Currently bioleaching is being used to recover metals, particularly Ag, Au, Cd, Co, Cr, Cu, Li, Mo, Ni, Pb, Sn, V and Zn from various industrial wastes. A significant increase in interest in this method is due to the fact that bioleaching is considered to be a low-input process and a technology that reduces the consumption of environmental resources and has a lower potential for the production of by-products. In addition, bioleaching is related to issues and implementing the circular economy. Most industrial waste containing valuable metals is a source of toxic pollutants. However, bioleaching is a method that enables the recovery of elements, but also eliminates their toxic properties [37]. A key issue for this technology is that typically "valuable" wastes do not contain energy sources for bioleaching bacteria such as iron ions and sulfur. In addition, the generally alkaline nature of the samples limits the growth of bacteria, so it becomes necessary to additionally acidify the samples. The aspect of proper adaptation of microorganisms and process conditions, temperature and reaction time of several days should also be mentioned [38,39].

The suggestion in the article to bioleach sludge from water purification, especially groundwater, seems reasonable. This is due to the possibility of producing good quality iron (waste) coagulant and biomass. This possibility is all the more rational as the mentioned products can be produced under low temperature process conditions (10°C, unheated reactors), shortened time (6–12 d) and without the required dosing of sulfur compounds as an energy substrate for bioleaching bacteria. An open analytical question is whether the gains from the production of biomass waste coagulant cover the losses (e.g., economy, carbon footprint) incurred for the necessary aeration of the reaction environment.

Also of interest are the G10 and G20 combinations based on anaerobic WPS preparation. The leaching efficiency of these combinations was only about 40%. However, it is also possible to recover matter – biomass, but also, for example, volatile fatty acids. Further research on WPS fermentation should also be carried out with a view to energy recovery, that is, the production of biogas by methane fermentation.

5. Conclusions

The tested substrate consisted mainly of iron post-coagulant deposits. Importantly, it was sludge from groundwater treatment and the content of heavy metals and organic substances did not indicate that this waste could cause significant environmental problems. It can therefore be concluded that this type of substrate requires less technologically advanced and less costly recycling methods and the resulting product is of good quality. In particular, it was considered justified to continue the experiments on bioleaching of metals. This was due to the possibility of recovery of matter in the form of both waste coagulant (iron salts) and biomass of bioleaching bacteria. However, this requires confirmation by further tests that will prove, firstly, the usefulness of the bioleaching liquid as a waste coagulant and, secondly, the possibility of utilizing the growing biomass of microorganisms for fertilization purposes. The technical aspect also seems to justify further research. Current research has confirmed that both metal bioleaching and biomass growth can take place at low temperatures, that is, in bioreactors that do not require heating. The disadvantage of the proposed technology is the need to aerate the bioreactor and the cultivation time. It is therefore to be expected that the carbon footprint analysis will show high carbon dioxide equivalent values. On the other hand, it can be assumed that the water footprint will decrease. An economic and environmental assessment is not possible at this stage of the research. Several issues need to be clarified. It was found that the tested sludge can be considered a good culture medium. An increase in biomass was observed for both samples inoculated with *A. thiooxidans* (combinations D, E, F) and non-inoculated (combinations A, B, C). Therefore, taxonomic studies seem to be necessary. Further work can be grouped in three areas of research. The first is the field of biotechnology, that is, the study of growth kinetics, productivity and efficiency of the bioleaching process. The second is reactor engineering and research such as oxygen demand and aeration methods. The third and most important area is technology and product valorisation issues. This includes aspects of possible purification and other ways of managing products other than regenerated iron coagulant and acid fertiliser biomass.

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