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Кафедра підземної розробки родовищ
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ПОЯСНЮВАЛЬНА ЗАПИСКА
дипломної роботи
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(назва освітньо-кваліфікаційного рівня)

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на тему: Оптимізація процесу вилугування мідних руд біологічно виробленими органічними речовинами (оцтова, лимонна та полі-глютамінова кислоти і амоній)

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2018

Міністерство освіти і науки України
Національний технічний університет «Дніпровська політехніка»

ЗАТВЕРДЖЕНО:
завідувач кафедри

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(підпис) (прізвище, ініціали)

«_____»_________________20___року

ЗАВДАННЯ
на виконання кваліфікаційної роботи магістра

спеціальності 184 Гірництво
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студенту 184м-16-11-ГФ Панков Ф. П.
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Тема дипломної роботи Оптимізація процесу вилугування мідних руд біологічно виробленими органічними речовинами (оцтова, лимонна та полі-глютамінова кислоти і амоній)

1 ПІДСТАВИ ДЛЯ ПРОВЕДЕННЯ РОБОТИ

Наказ ректора Державного НТУ "ДП" від __________________№_____

2 МЕТА ТА ВИХІДНІ ДАНІ ДЛЯ ПРОВЕДЕННЯ РОБІТ

Об'єкт досліджень процес вилугування руд на лабораторному стенді для оптимизації процессу.

Предмет досліджень дія мікроорганізмів на мінерали

Мета дослідження впливу мікроорганізмів варіюванням тривалості реакції, для визначення ефективності вилугування на породу та подальшої модернізації та автоматизації способів вилугування.

Вихідні дані для проведення роботи проведення експериментів на дослідницькому стенді в ТУ «Фрайберзька гірнича академія»

3 ОЧІКУВАНІ НАУКОВІ РЕЗУЛЬТАТИ

Наукова новизна полягає у встановленні залежності часу, концентрації діючих речовин, енергоспоживання від тривалість процессу вилугування.

Практична цінність полягає в розробці регресійних моделей, що дозволяють визначити залежність часу від еффективності процессу вилугування; розробці діаграмм математичної оптимизації та знаходження оптимальних параметрів процессу.

4 ВИМОГИ ДО РЕЗУЛЬТАТІВ ВИКОНАННЯ РОБОТИ

Обґрунтованість і достовірність наукових положень, висновків і рекомендацій, сформульованих в роботі, обумовлена: коректною постановкою завдань використанням фундаментальних положень хімічних та біологічних реакцій.

5 ЕТАПИ ВИКОНАННЯ РОБОТИ

Найменування етапів робіт	Термін виконання
Стан питання мета і задачі дослідження	16.10.17 – 21.10.17
Аналіз методів вилугування	22.10.17 – 03.11.17
Планування та проведення експериментів	04.11.17 – 10.12.17
Обробка даних	11.12.17 – 19.12.17
Аналіз та розрахунок ключових параметрів процесу вилугування	20.12.17 – 02.01.18
Розробка регресійних моделей та діаграмм оптимізації	03.01.18 – 21.01.18

6 РЕАЛІЗАЦІЯ РЕЗУЛЬТАТІВ ТА ЕФЕКТИВНІСТЬ

Економічний ефект застосування мікробіовилугування гірських порід дозволить знизити час вилугування в результаті перейти від хімічних ревгентів до біологічних, як результат оптимізувати безперервний процес вилугування.

Соціальний ефект застосування біовилугування дозволить відмовитись від хімічних реагентів, що є незаперечно забруднює навколишнє середовище токсичними елементами.

7 ДОДАТКОВІ ВИМОГИ

Відповідність оформлення згідно ДСТУ 3008-95. Документація. Звіти у сфері науки і техніки. Структура і правила оформлення.

Завдання видав _____________ Бондаренко В.І.
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Дата видачі завдання: 16.10.2017 р.

Термін подання дипломної роботи до ЕК 21.01.2018

Дніпро
2018

Declaration of Authorship

“I hereby declare that this thesis is entirely my own work except where otherwise indicated. The presence of quoted or paraphrased material has been clearly signaled and all sources have been referred. The thesis has not been submitted for a degree at any other institution and has not been published yet.”

Date …………………… Fedir Pankov ……………………

Acknowledgement

My Master thesis is being the greatest work that I have ever done by myself, therefore I would like to say "thank you" to many people!

First of all, I was lucky to participate in the study course "biotechnology in mining" held by Prof. Dr. Schlömann that in fact changed my mind what I am going to do in my professional life, and then agreed to be my official supervisor from the university.

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Also, small thanks goes to myself that I didn't give up and did my best, even though it was tough for me.

Abstract

The ore deposit of the European copper shale (kupferschiefer) is large sediment-hosted copper deposits with a high content of base metals, precious metals and PGM. The Copper shale ore possesses a large content of carbonate rocks up to 75% that hinders traditional bioleaching with acidophilic microorganisms. Therefore heterotrophic microorganisms were examined with respect to metals leaching at neutral pH to avoid carbonates dissolution.

In the present thesis optimization of the leaching process was investigated employing heterotrophic microorganisms to produce leaching agents as citric acid, acetic acid, ammonium and poly-γ-glutamic acid by Kombucha-culture, *Yarrowia lipolytica, Sporosarcina ureae and Bacillus licheniformis* respectively. Copper shale ore samples come that from Mansfeld mining waste dump (Germany), Sangerhausen mine (Germany) and Rudna mine (Poland) were leached with biologically produced leaching agents. Chemical analyses of ore samples demonstrate leaching kinetic and efficiency reaching copper yield up to 45.1%. Based on experiments planning technique and obtained results the response surface models were built in order to optimize the impute parameters such as temperature, pH and pulp density in order to increase copper yield.

Eventually, mineralogical analyses indicate copper minerals dissolution ratio of bornite, covellite, chalcocite and chalcopyrite giving an overview of on their ability to be leached at applied conditions. The results showed the dissolution of all copper sulfides that proof process feasibility.

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List of Abbreviation

w/v	weight per volume ration
BSE	back scattered electron
DSM	German collection of microorganisms
et al.	Latin for “and others”
g	gram(s)
PGA	poly-glutamic acid
PGM	platinum group metal
ICP-MS	inductively coupled plasma mass spectrometry
kV	kilovolt
l	liter
MeS	metal sulfide
min	minute(s)
ml	milliliter(s)
MLA	mineral liberation analysis
mm	millimeter(s)
µm	micrometre
pH	negative decadal logarithm of hydrogen ions, measure for acidity/alkalinity of liquids
pKA	negative decadal logarithm of ionization constant of an acid
PLS	pregnant leach solution
g	gravitational acceleration
rpm	rotations per minute
W	watt
ppm	parts per million

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1 Introduction

The permanent growth of the goods consumption requires more metals to be produced, meanwhile, the richest mineral deposits have been already mined (EUreport, 2014). That makes us take a closer look at the innovative way of metal extraction from the ore by cheaper techniques. Bioleaching is one of the greatest options but still needs to be adopted for various ore types (Kutschke, et al., 2015). Six polymetallic copper shale ore samples from Germany and Poland have been investigated in order to estimate recoverability of metals by means of bioleaching (Borg, et al., 2012). One of the main features of cooper shale ore is high carbonates content that leads to enormous neutralization potential when exposed to acidic conditions (d'Hugues, et al., 2008). Traditional bioleaching involves utilization of iron and sulfur oxidizing bacteria which require low pH (<3) but at the same time, low pH leads to carbonate dissolution and formation of precipitated gypsum that hinders copper minerals leaching (Kostudis, 2015). One of the opportunity is to keep the pH at neutral or alkaline but that is not supported traditional autotrophic microorganisms are not able to leach the metals at high pH. That leads to the selection of another type of microorganisms and in the present research four heterotrophic microorganisms are used to leach metals out of the ore. As long as heterotrophic bioleaching involves an indirect mechanism that means that metabolites produced during organic carbon consumption interact with minerals the main focus was put on the application biological produced components (Bosecker, 1997). The previous study showed an influence of heterotrophic bioleaching on metal dissolution from the ore but the recovery rates remained lower comparing to acidophilic leaching (Kostudis, 2015). In order to enhance metal yield, several microorganisms were used for the leaching experiments, such as *Bacillus licheniformis,* Kombucha-culture, *Yarrowia lipolytica* and *Sporosarcina ureae.* Optimization of the leaching was investigated varying pulp density, temperature and pH that allows the design of response surface model based experiment planning technique.

1.1 Metal scarcity and security supply

Metals establish a foundation for our present norm of living (Free, 2013) .

Modern society depends heavily on the products made of materials which come from mineral resources. However, every metal or chemical element possess various distribution around the word (Clarke, 1924), the areas with enriched minerals concentrations belong to extreme unstable, unstable, fair and stable countries (Reichl, 2016). Figure 1 shows the total mineral resources outcome from all around the globe according to their stability over 18 years span. Extreme unstable and unstable countries account for approximately 60-70% of the world mineral resources production.

Countries stability directly influences the metal prices and supply risks.

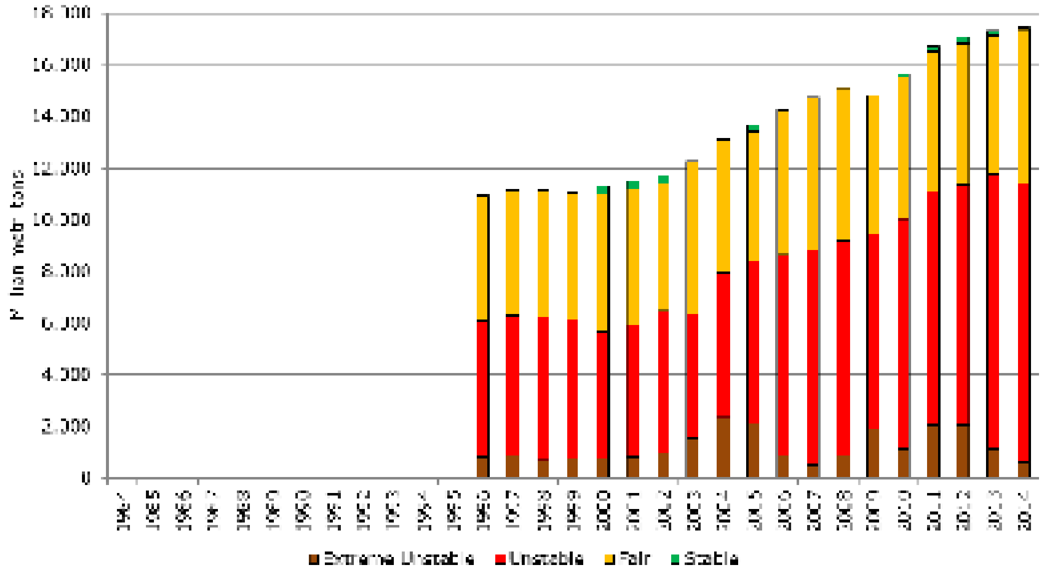


Figure 1 World mining production 1984-2014 by political stability of the producer countries (Reichl, 2016)

The amount of mining production from political fair or stable countries is less than 60 % of the annual production. The average production from fair or stable countries from 2006 to 2014 is around 40% whereas the production from extreme unstable and unstable increases in more than 3000 Mt from 14100 Mt to 17200 Mt.

In order to prevent sudden mineral resources cut off, new mineral deposits and old waste tailings are under investigation. Thus, processing techniques have to be developed with respect to mining and beneficiation costs reduction. As an availability of the approaches to extract mineral commodities extends from year to year, it became reasonable to estimate their applicability in the mining industry for various ore types depending on the current metals prices.

1.2 Metal prices volatility

European polymetallic copper shale ore contains a number of valuable metals such as V, Co, Cu, Zn, Ga, Pd, Ag, Pt, Au but traditionally Cu remains the main source of income (Borg, et al., 2012).

On Figure 2 demonstrated copper price alteration over 105 years with the main causes that lead to price change.

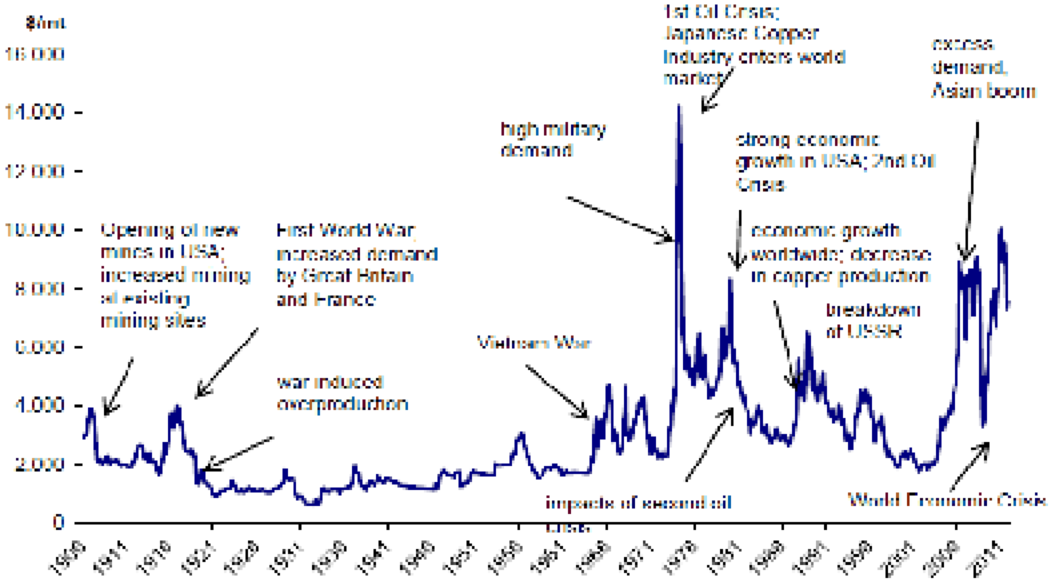


Figure 2 Reasons for price peaks, collapses and trends of mineral raw materials on the example of copper price change over 105 years (EUreport, 2014)

Depending on the occasions occurring around the word the metal prices can be dramatically changed within a short time. For instance, the copper price in 2001 was about 2000 $/metric ton, that could slow down the research on processing technologies development and implementation, but in 2006 when the price was about 9000 $/metric ton quite a number of copper mineral resources become profitable to explore and therefore, an extension of production facilities and new technologies implementation took place.

Calculation of metal values in the Polish copper shale (Table 1) from the Lubin mine is reported to be approximately 20 % higher for accompanying metals than the value of Cu on the year 2008 (Chmielewski, et al., 2009). Recalculation of the metal values with regards to the actual price on September 2016 shows Cu value reduction by approximately 75 % that make an industry take a closer look at the increase of co-product metal recovery in order to maximize the profit.

Table 1 Metals value in 1 ton of Lubin shale middlings (price comparison in 8 years)

Metal	Content, %	Metal price, US$/metric ton (21 February 2008)	Metal price, US$$/metric ton (07 September 2016)	Value, US$ (21 February 2008)	Value, US$ (07 September 2016)
Cu	2.7	8 050	4 607	217.3	124.4
Ni	0.0373	27 900	9 880	10.4	3.68
Co	0.0569	110 250	26 945	62.3	14.8
Pb	1.52	3 170	1 940	48.2	29.5
Ag	0.018	559 107	622 366	111.1	123.4
Zn	0.12	2 350	2 348	2.8	2.8

As the metal value altered over 8 years the changes in metal recovery preferences should be observed. For instance, on September 2016 copper price was almost two times less than in February 2008 and in the case of silver, an increase of the price was experienced by about 10% in 8 years, that makes values of copper and silver almost equal. Therefore, processing technologies development should take into account metals concentration as well as metals prices under current economic conditions in order to maximize the profit.

The obvious fact that there is a strong need for the recovery processes development of every valuable component depending on the current price.

1.3 Cooper supply and demand

Copper is a ductile, reddish metal, employed since the beginning of civilization, widely utilized due to unique properties such as high thermal and electrical conductivity along with ductility and corrosion resistance (Lossin, 2001). Copper is the 26th most abounding element in the earth's crust with the average content of 50 ppm (Habashi, 1997).

1.3.1 Supply and demand statistics

Whereas the world's biggest wide majority of copper mineral resources located in the Americas (Chile, USA, Peru and Mexico), Europe has a large polymetallic (Kupferschiefer) deposit with active operations in Poland (USGS, 2013).

Worldwide mine production of copper was 16 million tons in 2010 (Edelstein, 2012). Figure 3 presents that Chile is the largest copper producer, with over 5.4 million tons in 2010 that make up about one-third of world production. Together with Peru (8%), China (8%) and the USA (7%), the four biggest copper mining countries contribute to more than half of the world production. In recent decades there has been a steady increase in production in South America, essentially in Chile from 16% in 1985 to 34% of worldwide production now (USGS, 2013).

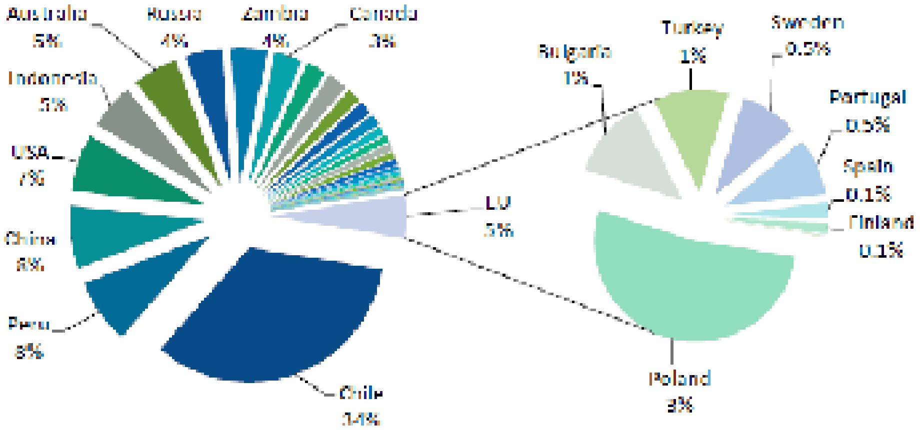


Figure 3 Worldwide production of copper in 2010 (EUreport, 2014)

EU-27 production of copper accounted for 814,277 tons in 2010, dominated by Poland which makes up over a half of the copper production in Europe (425,400 tons). As European copper production does not cover demand, the European Union is extremely dependent on import and recycling (EUreport, 2014)

There are over 150 identified copper minerals, with two of the highest importance: chalcopyrite and bornite. About half of world's copper production is produced from chalcopyrite (BGS, 2007). In Table 1 the most common primary and secondary minerals are indicated.

Table 2 The most important copper minerals (BGS, 2007)

Ore type	Mineral	Formula	Cu content, %
Primary sulfide minerals			
Sulphides	Chalcopyrite	$CuFeS_2$	34.6
	Bornite	Cu_5FeS_4	63.3
Secondary minerals			
Sulphides	Chalcocite	Cu_2S	34.6
	Covellite	CuS	79.9
	Digenite	Cu_9S_5	78.1
Native copper	Metal	Cu	100.0
Carbonates	Malachite	$CuCO_3 \cdot Cu(OH)_2$	57.5
	Azurite	$(CuCO_3)_2 \cdot Cu(OH)_2$	55.3
Hydroxy–silicates	Chrysocolla	$CuO \cdot SiO_2 \cdot 2H_2O$	36.2
Oxides	Cuprite	Cu_2O	88.8
	Tenorite	CuO	79.9
Sulphates	Antlerite	$CuSO_4 \cdot 2Cu(OH)_2$	53.7
	Brochantite	$CuSO_4 \cdot 3Cu(OH)_2$	56.2

1.4 Cooper ore mining

There are three principal techniques for copper mining: open-pit mining, underground mining and in-situ leaching.

Open pit mining is the most popular form and suitable for low-grade ores that are near to the surface. Underground mining is applicable for high-grade ores, e.g. these in the Rudna and Lubin mine Poland. With in-situ metal recovery, a leaching solution is pumped into the porous ore bodies to dissolve copper and then metal extracted from pregnant leach solution at the surface facilities. The Mopani mines in Zambia, Old Reliable mine (Arizona, USA), and the Zonia mine (Arizona, USA) are examples of the industrial use of in-situ leaching (EUreport, 2014).

1.5 European copper shale (kupferschiefer)

The ore deposits of the European copper shale is large sediment-hosted strata-bound copper deposits, located in Germany and Poland with active mining activity in Poland and after mining residues in Germany (Borg, et al., 2012). Figure 4 shows the operating mines and historical mining districts as well as the border of copper shale basin.

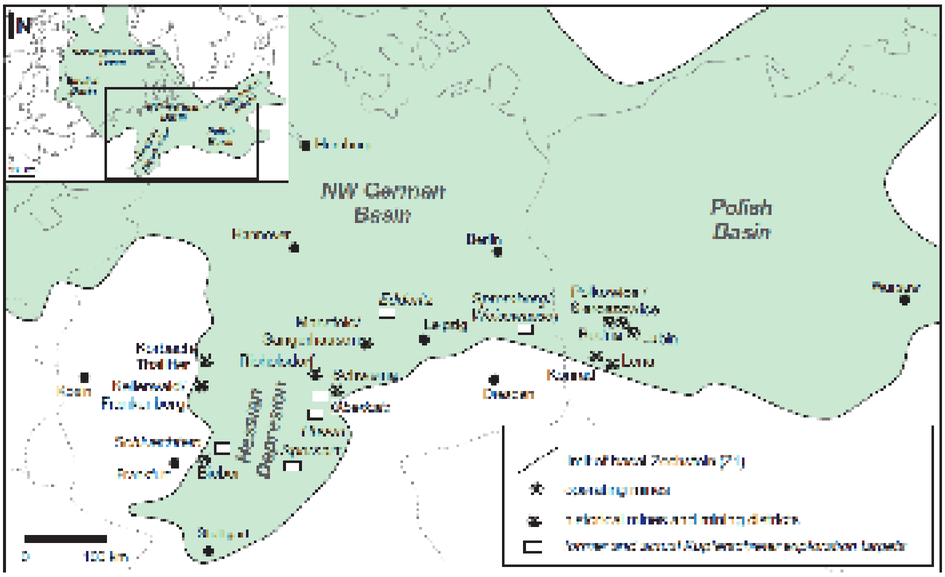


Figure 4 European copper shale basin with the various Cu mining districts and exploration areas (Borg, et al., 2012)

A map shows the borders of copper shale deposit with active mines and historical mining districts.

The copper shale mineralization proceeds to be a significant global producer of copper and silver with the locally high content of base metals (Pb, Zn, Co), precious metals and PGM (Borg, 2012; KUCHA, 1990)

The documented mining history of copper shale started in around 1200 in Mansfeld region (Germany). Ore processing technologies used in those time were focused on copper and provided incomplete metal recovery. Therefore, historical mining waste dumps still contain various metals (Cu, Zn, Pb, Ag, Au etc.) (Borg, et al., 2012). The area of Sangerhausen mineral deposit still possesses unexplored resources which are currently under the feasibility study due to metal price change and security supply risks.

Table 3 Copper and Silver Production and Current Reserves of Major copper shale Mining Districts of Germany and Poland (Borg, et al., 2012)

Period	District	Ore (Mt)	Cu metal (t)	Ag metal (t)
Germany				
1200 to 1990	Mansfeld			
	mined	80.76	2 009 800	11 111
	remaining reserves	none	none	none
	Sangerhausen			
	mined	28.14	616 200	3 121
	remaining proven reserves	35.40	860 000	4 650
Poland				
1949 to present	Mined, proven and indicated	>2 824	>55 452 894	
Total Germany and Poland				
	Mined plus remaining	>3 067	>60 427 894	

Mining of the Polish part of copper shale ore began at 1949 mostly by underground mining because of the high Cu content (up to 3.8%) and deep ore deposition.
Over last 25 years, annual production of ore accounts for about 26 Mt, from three main mines: Rudna, Polkowice-Sieroszowice, and Lubin (USGS, 2013). Ore grades typically in the limit of ca. 2% Cu and 50 ppm Ag. Furthermore, there are important by-products, such as Pb, Zn, Au, Ni, Pt, Pd, Re, and Se (KUCHA, 1990). In Table 3 listed mining areas with indicated ore resources and reserves according to JORC code, which shows already mined and remaining Cu metal within the European copper shale.

The Copper shale ore possesses a large content of carbonate rocks up to 75% (Chmielewski, et al., 2009) that lead to high neutralizing potential during leaching under acidic conditions. Traditional methods of metals recovery such as acid leaching and bioleaching with acidophilic microorganisms hindered by the precipitation of formed gypsum that leads to an investigation of the leaching at neutral or alkaline conditions (Kutschke, et al., 2015).

1.6 Hydrometallurgy

The term hydrometallurgy consists of prefix "hydro" which indicates water, and its suffix "metallurgy," which means the study of metals. Therefore, hydrometallurgy is a study of the interaction between water and metals (Free, 2013). Hydrometallurgy includes reacting the ore at low or moderate temperatures with a liquid solvent that selectively dissolve the valuable minerals. Separating the dissolved metals from the pregnant leach solution (PLS) by chemical (electrochemical) means, concentrating and purifying the desired metal cause formation of the pure metal (Bautista, 1984).

1.6.1 Leaching

The primary action in the hydrometallurgical treatment of ore is leaching. During this step, the aqueous reagent is utilized to the ore rock to ionize the metal and put it into solution. Leaching can be implemented to ore:

- **Stacked in heaps**

Ore crushed to appropriate particle size, placed in the large heaps and sprinkled with lixiviant which then collected at the bottom of the heap. Heap leaching used in case of low-grade ores and process run up to several years.

- **In vats**

Vats are used for high-grade ore, where the grounded ore reacts with the leaching agent under constant mixing. This type of leaching characterized by relatively high leaching kinetics, usually, process takes from several hours to a couple of days.

- **Under the pressure in an autoclave.**

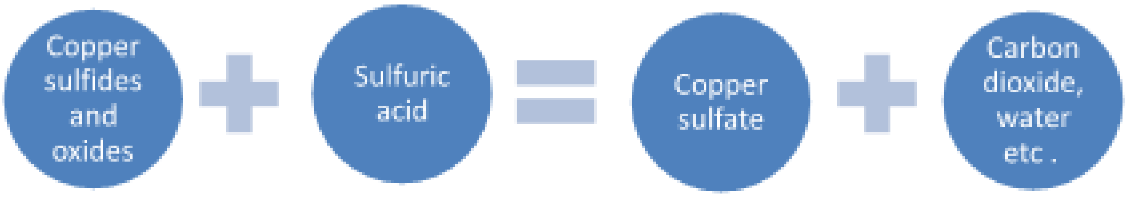
Pressure supports the refractory minerals oxidation under the elevated temperature that accelerates the leaching process, bringing down leaching time to some hours.

Any mineral can be dissolved using an appropriate leaching agent, but the most common applications are for copper and gold ores (Dunbar, 2015). Numerous chemical compounds are applied in order to dissolve minerals and form stable complexes or metal ions which remain in solution until extraction stage.

There are some conventional techniques for base metal (Cu, Ni, Co, Zn etc.) leaching.

1.6.2 Acid leaching with sulfuric acid

Sulfuric acid (H_2SO_4) is applied to low-grade copper sulfides and oxides (from the secondary sulfide zone in a copper deposit) as illustrated in the following scheme:



The result is a solution of copper sulfate and a number of different compounds, depending on the particular sulfide or oxide. Some of these reactions proceed slowly or use large amounts of acid (Dunbar, 2015; Free, 2013).

1.6.3 Leaching with ammonia

Ammonia has been proofed as efficient leaching agents in hydrometallurgical processes due to low toxicity and cost, simple recovery and great selective recovery of metals.

Ammonia leaching was first used for recovery of nonferrous metals, but the use of this technology was advanced from traditional nonferrous metals such as copper, nickel and cobalt to the extraction of zinc, cadmium, silver and gold (Radmehr, 2013).

Leaching employing ammonia results in the formation of soluble, strong stable ammonia complexes ($[Cu(NH_3)_4]^{2+}$). The major advantage of ammonia use in hydrometallurgical processes is selective solubilisation of desired metals and precipitation of undesirable iron (Han & Meng, 1996)

Ammonia leaching is applied in non-oxidative, oxidative and reductive leaching and can be utilized at ambient temperature due to the great solubility of ammonia in water. (Chmielewski, et al., 2009)

1.6.4 Biohydrometallurgy

Biohydrometallurgy is a field that incorporates the overlapping areas of biology and hydrometallurgy. Microbial activity is associated with a wide diversity of reactions linked to the mineral formation and degradation that have occurred over millions of years. The variety of biological reactions and their relations with metals in mineral and metals in dissolved forms has followed in the use of microorganisms in a broad range of metal extraction, metal recovery, and water treatment applications (Free, 2014).

1.6.5 Bioleaching with iron and sulfur oxidizing bacteria

In the case of bioleaching of metal sulfides they are oxidized to metal ions and sulfate plus intermediate sulfur compounds in acidic solution by aerobic, acidophilic $Fe(II)^{-}$, and/or sulfur compound oxidizing bacteria or archaea as shown in Figure 5 (Scheper, et al., 2014). Metal sulfides bioleaching differentiate two main dissolution mechanisms for acid soluble and acid non-soluble minerals. Thiosulfate mechanism involves Fe^{3+} attack in order to dissolve metals from the ore, meanwhile, polysulfide mechanism employs H^{+} attack on the mineral surface and cause acid formation.

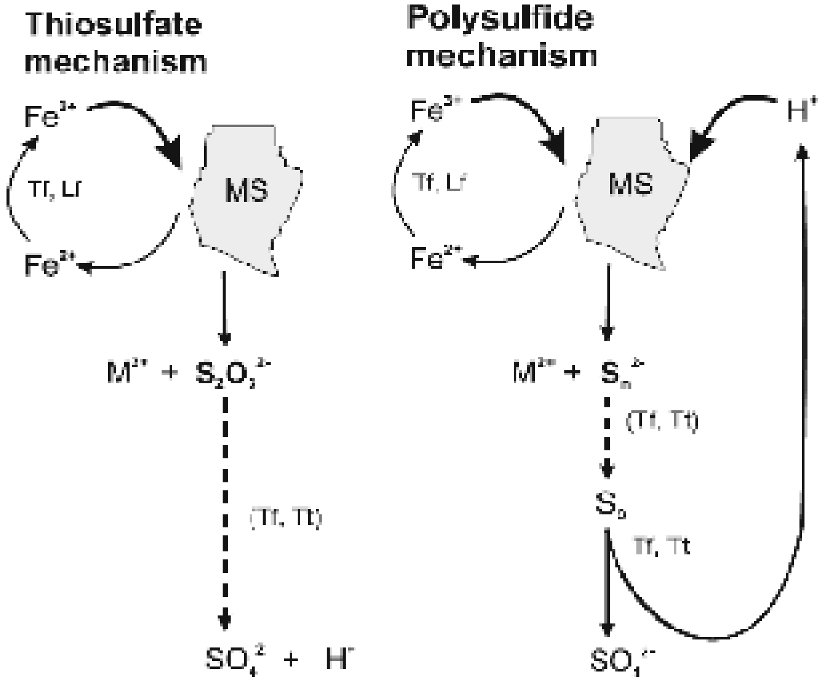


Figure 5 Bioleaching proceeds by two different indirect mechanisms.

Thiosulfate and polysulfide ore leaching mechanisms based on the properties of metal sulfides (MS). Dashed lines indicate the occurrence of intermediate sulfur compounds (Schippers & Sand, 1999)

There are bacteria that usually occur in the leaching environment and contribute to bioleaching process to a great extent, for example, *Acidithiobacillus ferrooxidans* and *Leptospirillum ferriphilum*.

Pyrite dissolution accelerated by the presence of microorganisms, release ferrous iron and sulfur to the solution.

The oxidative compound Fe(III) for the metal sulfides oxidation is provided by the microbial Fe(II)-oxidation, meanwhile, sulfur components are oxidized by the microorganisms to sulfate (Scheper, et al., 2014).

1.6.6 Heterotrophic bioleaching

Some heterotrophic bacteria and fungi are recognized for their capacity to leach elements, particularly from carbonaceous materials. (Scheper, et al., 2014). But in contrast to acidophilic bacteria and fungi, heterotrophic microorganisms obtain their energy and cell carbon from organic carbon sources such as sucrose. Heterotrophs leach minerals by providing acids such as citric, formic, acetic, glucuronic, oxalic, lactic, pyruvic, succinic etc. acids that are produced as by-products of organic carbon metabolism. (Free, 2014)

Leaching with heterotrophic microorganisms generally utilizes an indirect mechanism of organic acids, amino acids and other metabolites production. Those metabolites dissolve metals from minerals by displacement of metal ion from the ore by hydrogen ions or by the formation of soluble metal complexes and chelates. (Rezza, et al., 2001).

Indirect mechanism of heterotrophic leaching means that formed products of bacteria metabolism react with minerals (Bosecker, 1997). Therefore, understanding of heterotrophic leaching processes requires knowledge of minerals interaction with particular produced metabolite.

Basically, application of heterotrophic microorganisms is promising in terms of leaching agents production nearby processing facility using organic carbon sources as a growing media. Heterotrophs should be able to substitute utilization of chemically produced lixiviants.

Nevertheless, due to the variety of excreted leaching substances, the enormous amount of possible bioleaching microorganisms, as well as a wide pH range, makes leaching with heterotrophs attractive for the recovery of important metals or the beneficiation of minerals (Scheper, et al., 2014).

In the present study were used *Bacillus Lichenifotmis*, *Sporosarcina ureae*, *Yarrowia lipolytica* and Kombucha-culture in order to produce poly-γ-glutamic acid (PGA), ammonium, citric acid and acetic acid respectively.

1.7 *Bacillus Lichenifotmis*

The genus Bacillus is Gram-positive, rod-shaped bacteria (Figure 6). Physiologically bacteria are heterogeneous, and at least six main groups acknowledged based on phenotypic characteristics and rRNA sequences (Shariati, et al., 1995).

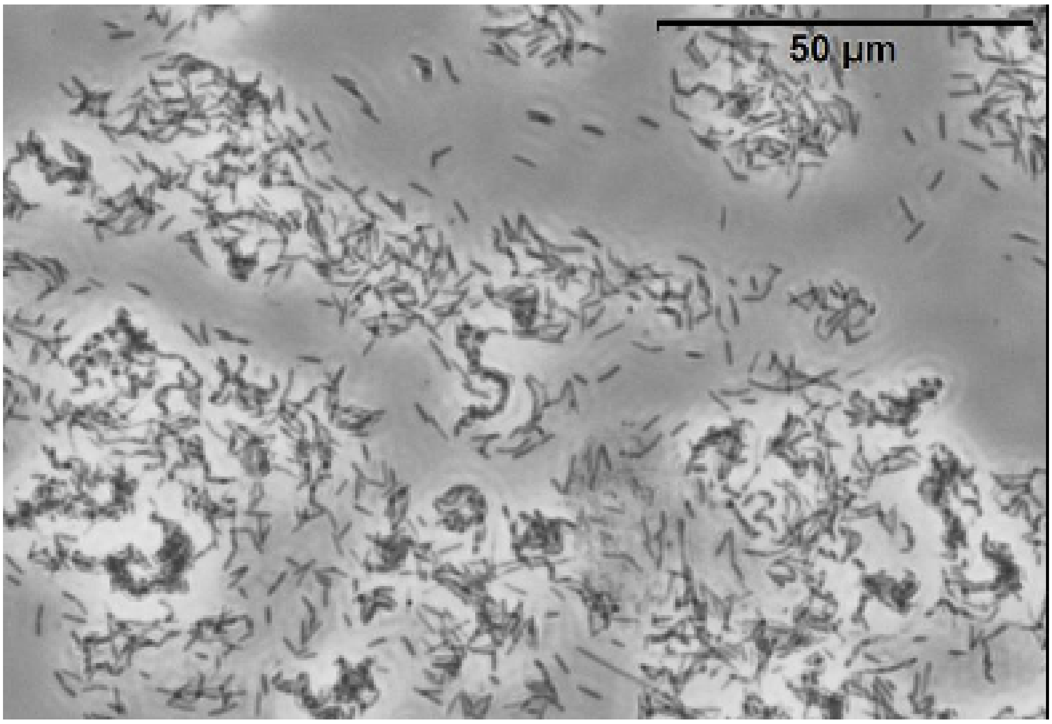


Figure 6 *B. licheniformis* DSM 8785 bacterial cells during the cultivation

Bacillus licheniformis provides various important industrial products, nowadays PGA seems to be the most important source of bacteria metabolism (Priest, 1993). An understanding of the physiology of this micro-organism, mainly its growth response to alterations in culture redox potential, is consequently critical in the optimization of industrial fermentations. (Shariati, et al., 1995)

1.7.1 Poly-γ-glutamic acid

Poly-γ-glutamic acid is a biodegradable polymer composed of L- and/or D-glutamic acid linked by an amide linkage between a-amino and g-carboxyl residues (Figure 7). The polymer is synthesized by various Bacillus species through a fermentation process. Since PGA has various important characteristics such as water solubility, biocompatibility and non-toxicity, therefore, PGA has gained recognition as the base material for many industrial applications (Mitsunagai, 2016).

One of the most important properties of PGA for mineral processing and water treatment industries is an ability of metal ion binding (Ni_2^+, Cu_2^+, Mn_2^+, and Al_3^+) under neutral or alkaline conditions (Chang, et al., 2013).

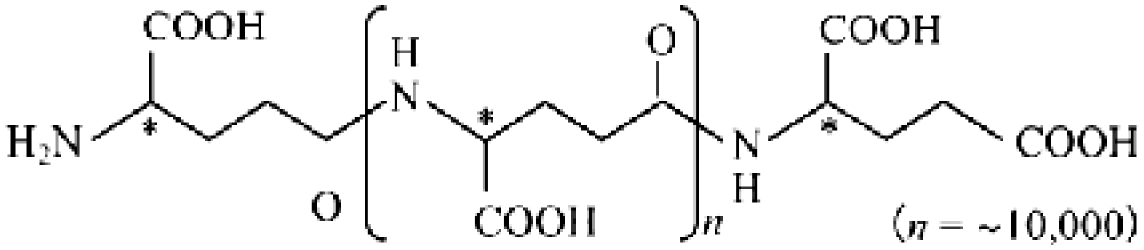


Figure 7 Molecular structure of PGA (Ashiuch, 2013)

After an interaction between PGA and metal ions, the metal ions form complexes, attaching to the PGA surface under neutral or slightly alkaline pH that promotes metal extraction from the solution (Margaritis, 2007).

In the present study investigated the interaction between copper minerals and PGA with respect to metal extraction from the ore.

1.8 *Sporosarcina ureae*

Being reported from (Groudeva, et al., 2007) that ammonium producing bacteria are able to dissolve copper minerals, *Sporosarcina ureae* DSM 2280 is chosen to produce ammonium for bioleaching experiments.
Sporosarcina ureae motile, spore forming coccus (Figure 8), possesses the enzymes required for a functioning urea cycle (Goldman, et al., 1988)

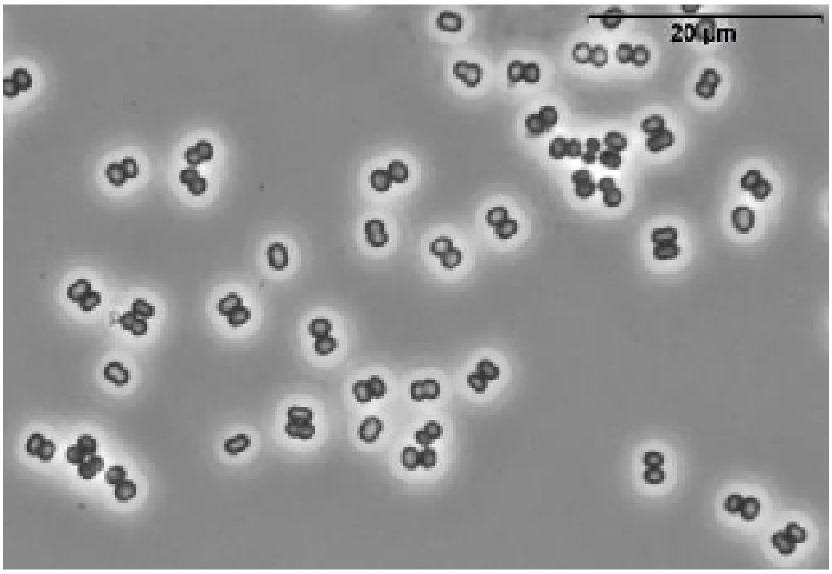


Figure 8 Sporosarcina ureae DSM 2280 bacterial cells during the cultivation

In environments rich in arginine, degradation by arginase provides intermediates capable of accessing the citric acid cycle. Urea can thus become a nitrogen source (ammonia) for further amino acid synthesis. (Goldman, et al., 1988)

Ammonia resulting from the hydrolysis of urea provides an alkaline pH. Alkaline bioleaching in the presence of ammonia producing microorganisms is promising for some ore types when traditional bioleaching inhibited by acid soluble gangue minerals (Radmehr, 2013).

1.9 Kombucha-culture

Tea fungus also known as kombucha is the common name for a symbiotic growth of acetic acid bacteria (*Acetobacter xylium, Acetobacter xylinoides, Bacterium gluconicum, Acetobacter aceti, and Acetobacter pasteurianus*) and osmophilic yeast strains (*Schizosaccharomyces pombe, Saccharomyces ludwigii, Kloeckera apiculata,* Saccharomyces cerevisiae, Zygosaccharomyces rouxii, Zygosaccharomyces bailii, Brettanomyces bruxellensis, Brettanomyces lambicus, Brettanomyces custersii, Pichia membranaefaciens, Torulopsis, and Candida) which should be cultivated in sugared tea. (Jayabalan, et al., 2010)
Acetic acid and gluconic acid are the main metabolites of the Kombucha-culture broth (Liu & Chen, 2000).That is being an important property in terms of unusual industrial application such as leaching process.

Acetic acid (CH_3COOH) is an organic acid with a pK_a value of 4.76 in an aqueous solution that is able to serve as a chelating agent (Oustan, et al., 2011).
Gluconic acid ($C_6H_{12}O_7$) is a noncorrosive, non-volatile, non-toxic, mild organic acid. It is a good chelator at alkaline pH with pK_a 3.7 (FU, et al., 2014).

The Kombucha-culture study revealed that the composition of tea fungus metabolites in the culture broth varied in various fermentation time points. Thus, it shows the possibility to obtain the desired quantity of organic acids in the tea fungus broth by regulating the fermentation conditions (Liu & Chen, 2000)

1.10 *Yarrowia lipolytica*

Yarrowia lipolytica is a heterothallic yeast that usually isolated from dairy products, but also from marine and hypersaline environments (Nicaud, 2012). Additionally, it has been observed that Y. lipolytica can secrete several kinds of metabolites in large quantities, such as organic acids, in a case of present thesis work, citric acid was estimated to be the main component (Knutsen, et al., 2007)
Citric acid is a weak, non-toxic tetradentate chelating agent with pK_a value 3.13 has been proved to be a good chelating agent in aqueous alkaline media (MULLER, et al., 1997).

Most *Yarrowia* strains are incapable of growing above 32°C and the species is strictly aerobic, therefore considered as non-pathogenic citric acid producer (Nicaud, 2012). In recent years, the non-conventional yeast *Yarrowia lipolytica* was employed in a wide variety of industrial applications, such as organic acid production, protein production and bioremediation (Suna, et al., 2016).

2 Materials and methods

2.1 Preparation of representative samples

The experiments were performed with samples from the Rudna mine of KGHM in Poland, from Mansfeld mining relics and from Sangerhausen. The run of mine samples from Runda mine were described as sandstone, Kupferschiefer, and carbonate. The used specimen from Sangerhausen was Kupferschiefer, the samples from Mansfeld mining district were divided by hydrocyclone and divided into two fractions Mansfeld slime (<50 μm) and Mansfeld deslime (-90+50 μm).

The Kupferschiefer run of mine ore samples were crushed and ground with a ball mill (McCrone, USA) by UVR-FIA GmbH. Ground ore samples with the average weight of 1.5 kg were stepwise split up by six times in every cycle with a sample divider (Retsch, Germany). The obtained samples possess the weight of 2 x 250 g, 4 x 50 g and 30 x 2.5 g.

2.2 Chemical analyze of ore samples

In order to obtain the chemical content of the feed ore, before ICP-MS measurement, solid material must be completely digested with the aid of strong acids.
For this purpose samples with the weight of 0.05 g ± 0.005 g were digested in 2 steps.

The first composition includes a mixture of solid material, 3 ml HNO_3 (65%), 1 ml HF (48%) and 1 ml HCl (32%). Digestion takes place in the multiwave (Anton Paar, Austria) over 20 min span. The entire cycle takes 20 min, after heating up the mixture remains at a temperature of 175°C, the maximal power of 1000 W and pressure of 7*106 Pa for 10 min and then cooled down.
The second step includes an addition of 7 ml H_3BO_4 (5%) and the same digestion procedure takes place. Cooled down solution was diluted with 15 ml of osmosis water. Completely dissolved samples were used for ICP-MS measurement.

2.3 Production of poly-γ-glutamic acid by *Bacillus licheniformis*

For the γ-PGA production *Bacillus licheniformis* strain DSM 8785 from the Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures was used. For cultivation of *B. licheniformis* DSM 8785, optimized Medium E (Preißler, 2016) with the following components concentration shown in Table 4 has been used.

Table 4 Opitmized Medium E

Components	M [g/mol]	**concentration** [g/l]
Citric acid	192.13	18
Glycerol	92.09	120
NH4Cl	53.49	21
K2HPO4	174.18	0.5
MgSO4 x 7 H2O	246.48	0.5
FeCl3 x 6 H2O	270.69	0.04
CaCl2 x 2 H2O	147.02	0.15
MnSO4 x H2O	169.02	0.104
Purified water		1000 ml

The cultivation of *B. licheniformis* DSM 8785 was carried out under controlled conditions in the 5l bioreactor (Applikon, The Netherlands), the cultivation demonstrated in Figure 9. After the bioreactors had been prepared accordingly, they were filled with the appropriate medium and then the reactors were autoclaved and connected to the control systems. Using the associated software, parameters such as pH, temperature and the percentage of dissolved oxygen controlled and documented. Cultivation was carried out under sterile conditions at 37°C with a minimum dissolved oxygen concentration of 30 %. The pH adjusted after the lag phase to 6.5. The solution supplied with 6.68 l/min of air.

Figure 9 γ-PGA production by *B. licheniformis* DSM 8785

Bioreactor (left-hand side) filled with Medium E until bacteria culture inoculation, bioreactor (right-hand side) after inoculation with bacteria culture (70 hours).

The culture broth was harvested via centrifugation (Thermo Scientific Sorvall® Evolution™) for 30 min with the acceleration of 13000 g, at 4°C. Supernatant without bacteria cells later was stored in the freezer at -20°C until the utilizing in the leaching experiments.

2.4 Quantitative determination of γ-PGA with CTAB-assay

In order to determine the amount of formed γ-PGA during cultivation, the method of (Ashiuchi & Makoto, 2011) was employed. The CTAB assay is a photometric method for the quantitative determination of γ-PGA.
After presumption that the maximal concentration of γ-PGA is reached during cultivation (Preißler, 2016), samples were taken three times to make sure that no increase in γ-PGA is observed. For measuring cell-free samples were used, therefor the culture broth was centrifuged for 10 min with 20 000 g acceleration at 4°C. Then, cell-free supernatant was diluted in order to get the values in the detection limit. One ml of the supernatant was mixed with 0.2 ml 0,.1 M cetrimonium bromide (CTAB). A reaction takes 20 min before spectrometrical analysis (wavelength 400 nm) (Ashiuchi & Makoto, 2011).

Obtained values show light absorption by γ-PGA and further recalculated according to previously recorded calibration curve (Preißler, 2016), which leads to determining the concentrations of the respective samples.

The measured absorbance showed the concentration 12-13 g/l of γ-PGA.

2.5 Production of Ammonia by *Sporosarcina ureae* DSM 2280

The cultivation of *Sporosarcina ureae* DSM 2280 was carried out under controlled conditions in the 5 l bioreactor (Applikon, The Netherlands). For the NH_4^+ production, *Sporosarcina ureae* strain DSM 2280 from the Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures was used.
The cultivation was carried out in the nutrient broth (Mast Group, United Kingdom) with concentration 10 g/l.

After the bioreactors had been prepared accordingly, they were filled with the appropriate medium and then the reactors were autoclaved and connected to the control systems. Using the associated software, parameters such as pH, temperature and the percentage of dissolved oxygen controlled and documented. Cultivation was carried out under sterile conditions at 30°C, with a minimum dissolved oxygen concentration of 30 %. The pH was adjusted and controlled to 7 at the beginning of cultivation. The solution was supplied with 6.68 l/min of air. Cultivation took 48 hours then the culture broth was harvested via centrifugation (Thermo Scientific Sorvall® Evolution™, USA) for 30 min with the acceleration of 13000 g, at 4°C.
Supernatant without bacteria cells later was stored in the freezer at -20°C until the utilizing in the leaching experiments.

2.5 Ammonia assay

The concentration of ammonia produced by *S. urea* DSM 2280 was performed according to the instruction of Megazyme®Ammonia (Rapid) K-Amiar Assay Procedure (Megazyme, Bray, Ireland) ammonium determination kit.

In the presence of glutamate dehydrogenase (GlDH) and reduced nicotinamide-adenine dinucleotide phosphate (NADPH), ammonia (as ammonium ions; $NH4^+$) reacts with 2-oxoglutarate to form L-glutamic acid and NADP.

$$\text{2-Oxoglutarate} + \text{NADPH} + NH4^+ \longrightarrow \text{L-glutamic acid} + \text{NADP} + H2O$$

The amount of NADP+ formed is stoichiometric with the amount of ammonia. The absorption was measured at λ = 340 nm.
Determining the absorbance difference (A_1-A_2) for both blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $_\Delta$A ammonia (Megazyme, 2016). The concentration of ammonia can be calculated as follows:

$$C = \frac{V * MW}{\varepsilon * d * V} * {}_\Delta A$$

where:
V = final volume [mL]
MW = molecular weight of ammonia [g/mol]
ε= extinction coefficient of NADPH at 340 nm= 6300 [$l \times mol^{-1} x\ cm^{-1}$]
d = light path [cm]
v = sample volume [mL]

2.6 Leaching experiments design

In order to show an impact of biologically produced poly-glutamic acid (γ-PGA) on metal liberation from the Kupferschiefer ore leaching experiments under various conditions were performed.

Three main parameters such as pulp density, temperature and pH were chosen to alter during the experiment.

Optimization of metal yield requires experiments planning, therefore Box-Behnken experiments design method was applied (Table 5). This method allows determination of the most influencing parameters. Processing obtained data, the statistical presumption can be made revealing optimized parameters which should increase the metal yield. Experiments results were processed with analytical software (Quantum XL Software for Microsoft® Excel®).

The response surface model is an outcome, which illustrates dependence between leaching conditions and copper yield (Cavazzuti, 2013).

Table 5 Designed experiments plan by Box-Behnken method with 3 variables.

№	Pulp density	Temperature	pH
1	10	27	6.5
2	10	37	6.5
3	20	27	6.5
4	20	37	6.5
5	10	32	5
6	10	32	8
7	20	32	5
8	20	32	8
9	15	27	5
10	15	27	8
11	15	37	5
12	15	37	8
13	15	27	6.5

2.6.1 Leaching with γ-PGA

The leaching experiments were performed in Erlenmeyer flasks. In order to prevent random error, two samples with the same content were running in parallel.

Adjustment of initial pH in the leaching solution carried out by stepwise addition either 10 M NaOH or 10 M H_2SO_4.

Initial pulp density with the weight per volume ratio (w/v) of 10, 15, 20 adjusted by addition of 25, 16.7 and 12.5 ml respectively of supernatant produced by *B. licheniformis*.

Incubator Hood (Edmund Bühler GmbH, Germany) is set to 120 rpm for all the experiments with the temperature of 27°C, 32°C and 37°C. Erlenmeyer flasks were placed inside the incubator hood during ten days of each experiment.

Performing the tests in Erlenmeyer flasks, evaporation took place that notably influenced the results comparison. At the end of leaching experiments, the amount of evaporated water was detected and included in calculations.

2.6.2 Leaching with stepwise change of supernatants

In order to boost the cooper yield, the following experiment with pre-treatment steps was set. Weak acids and ammonia were utilized before the leaching with γ-PGA.

The experiment was carried out in the overhead reactor under the lab temperature (average 22°C) and pulp density 15% (w/v).

In the blue box, a microorganism is indicated which was used to produce particular metabolite.

Cell-free supernatants of *Kombucha* and *Yarrowia lipolytica* (6.5 g/l of citric acid) with defined characteristics were supplied from previous projects.

After every 10 days of leaching, the solid material was washed out with deionized water and then, the next cell-free supernatant was added (Figure 10).

The duration of every experiment is 10 days.

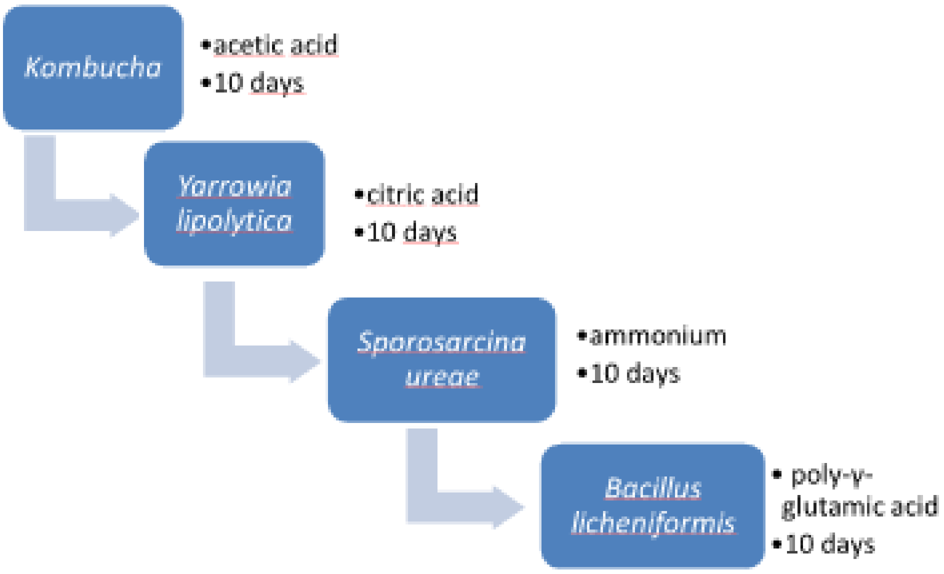


Figure 10 Scheme of the leaching experiment with stepwise change of supernatants

In the blue box indicated microorganism which was used and right side of the box the metabolite is indicated.

Leached ore samples afterward were collected for mineralogical analysis by X-ray Powder Diffraction (XRD) and Mineral Liberation Analysis (MLA).

2.7 Sampling procedure

The samples of the pregnant leach solution were taken on the 2nd, 4th, 7th and 10th day of each experiment.

The suspension with the volume of 0.35 ml from every Erlenmeyer flask was centrifuged for 10 minutes with the acceleration of 20 000 g to precipitate the solid matter. Figure 11 shows pregnant leach solution (PLS) of experiment with 15% w/v pulp density, pH=6.5, and temperature 32°C after 10 days. An aliquot of supernatant was stabilized with 2.5 µl of HNO_3 (63%) and analyzed by ICP-MS.

Samples were stored at 4°C until analysis. If precipitates were formed, prior analysis the samples were heated to 80°C.

1.Mansfeld	2.Mansfeld	3.Sangerhausen	4.Rudna	5.Rudna	6.Rudna
Coppershale	Coppershale	Coppershale	Sandstone	Shale	Carbonate
(deslimed)	(slime)				

Figure 11 PLS of Kupferschiefer ore samples after 10 days of leaching with γ-PGA

ICP-MS results, first of all, were recalculated in order to illustrate how much of the metal from the feed ore was dissolved.

$$\beta = \frac{x * V}{m * 10000}$$

β – calculated particular metal content in the PLS out of the feed ore (%)
x – metal ions concentration in the PLS, according to ICP-MS (µg/L)
V – volume of the PLS (L)
m – weight of ore sample (g)

The yield characterizes the effectiveness of the leaching process, demonstrating how much of the metal was extracted in relation to the feed ore.

$$\gamma = \frac{\beta * 100}{\alpha}$$

γ – yield, %
α – metal content in the feed ore, %

The metal content of two parallel experiments deviated in the limits of 3%. The results of the parallel tests were summed up and divided by 2 in order to obtain an average value.

2.7.1 Mineralogical analyses

Ore samples were analyzed by X-ray diffraction (XRD) and mineral liberation analysis (MLA). Two different methods were used to prevent a case of totally wrong mineral identification All measurements were performed by the analytical department of Helmholtz Institute Freiberg.

The results of mineralogical analyses of the feed ore samples were provided before at the beginning of experiments.
In order to estimate leaching efficiency, the remaining ore samples after the experiments with a stepwise change of leaching supernatants were handed over to the analytical department.

An important feature that ore samples were weighted before and after leaching and then obtained mineralogical results were recalculated taking into account dissolved minerals.

$$\boldsymbol{Mineral\ content\ after\ leaching} = \frac{\boldsymbol{m1} * \mathbf{R}}{\boldsymbol{m2}}$$

Where:

$\boldsymbol{m1}$ - weight of ore sample after leaching (g)

$\boldsymbol{m2}$ - weight of ore sample before leaching (g)

$\mathbf{R}$ - result of mineralogical measurments in the leached ore

That enables mineral content comparison taking into account all dissolved minerals.

3. Results

The goal of the present thesis was to establish an optimization of the leaching process with respect to metal recovery from copper-bearing ore samples by means of biologically produced leaching agents. The yield of various elements was measured but as long as copper is being one of the most valuable metal in the ore and the copper yield was much greater comparing to the other elements, the results are dedicated to copper extraction. The major focus was put on the utilization of PGA as a leaching (complexing) agent for metal extraction, therefore Box-Behnken experiments planning technique was implemented in order to enhance copper yield. Processing obtained results of leaching experiments enables the further optimization of metals yield and was successfully applied to Mansfeld copper shale (deslimed hydrocyclone product), Mansfeld copper shale (hydrocyclone slime) and Rudna Shale presuming the increase by 1.64%, 3.33% and 1.4% of copper yield respectively. Then, experiments with a stepwise change of supernatants were set under defined conditions. Which showed an increase in the copper recovery for Mansfeld copper shale (hydrocyclone slime) and Rudna Carbonate ore by 4.5% and 1.6% respectively samples but the most important fact that almost the same yield of copper was reached using weak organic acids and ammonium as a pre-treatment may be interpreted in a various manner. One of the reasons can be a presence of refractory copper ore minerals that remains insoluble. That led to a mineralogical analysis of the ore samples and discussions how to overcome this issue.

In order to estimate an influence of leaching compounds, ore characteristic such us change in mineral composition and elemental content before and after experiments were measured to demonstrate an efficiency of the process. Copper minerals possess various ability to be dissolved under a certain condition such as temperature, pH, pulp density, presence of the complexing agents etc. In order to track whether minerals were dissolved or new minerals were formed, mineralogical analyses by XRD and MLA techniques were performed and the results are indicated in graphs for each ore sample. The abundance of gangue such as carbonates, clays, feldspars and quartz much rather characterized by XRD measurements. At the same time, in terms of Copper sulfides, quantification MLA gives more reliable results even though they are overestimated due to quartz and clays underestimation, but provides relative proportion between sulfide minerals.

Mineralogical analyze of gangue shows main features of the ore due to special properties of the mineral groups. Carbonate minerals possess high neutralization potential that causes its dissolution during acid treatment, clay minerals usually characterized by small particle size that can influence the pulp properties in the leaching process, quartz minerals possess high hardness and therefore the particles are course after crushing and grinding that influence pulp property and particle size distribution. These gangue minerals properties were considered before particular leaching agent application.

3.1 Mansfeld copper shale (deslimed)

Gangue content characterizes the ore properties and considered to be an important factor which influences the leaching process.

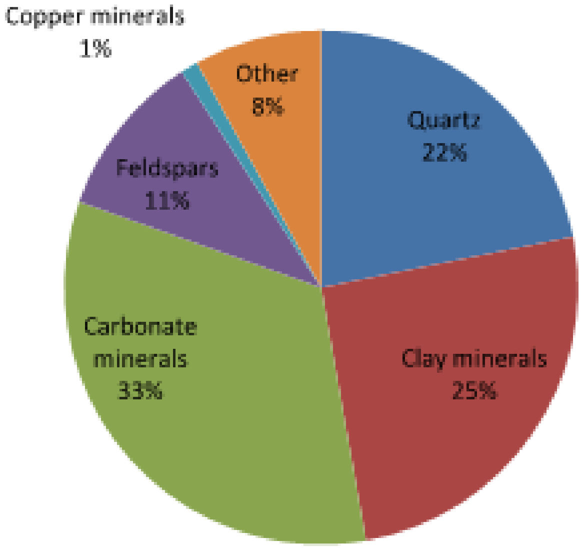


Figure 12 Mineralogical content of Mansfeld copper shale (deslimed)

The pie chart shows the main groups of mineral in the Mansfeld Copper shale, deslimed hydrocyclone product (-90+50µm) that describes the gangue content that was measured by XRD

Figure 12 demonstrates the mineralogical content of the main groups of minerals such as carbonates, clays, quartz, feldspars and metal sulfides measured by XRD for Mansfeld copper shale (deslimed hydrocyclone product).

The largest group of minerals is carbonates which make up 36% of the total weight. Clay minerals and quartz distributed in the ore sample almost equal and account for 25% and 22% respectively. A minor group of gangue is feldspars with the 11% of the weight. The feed ore contains about 1% of copper ore.

3.1.1 Leaching with PGA

Figure 13 illustrates leaching results with the highest copper yield among the experiments using PGA as a leaching agent for Mansfeld copper shale (deslimed).

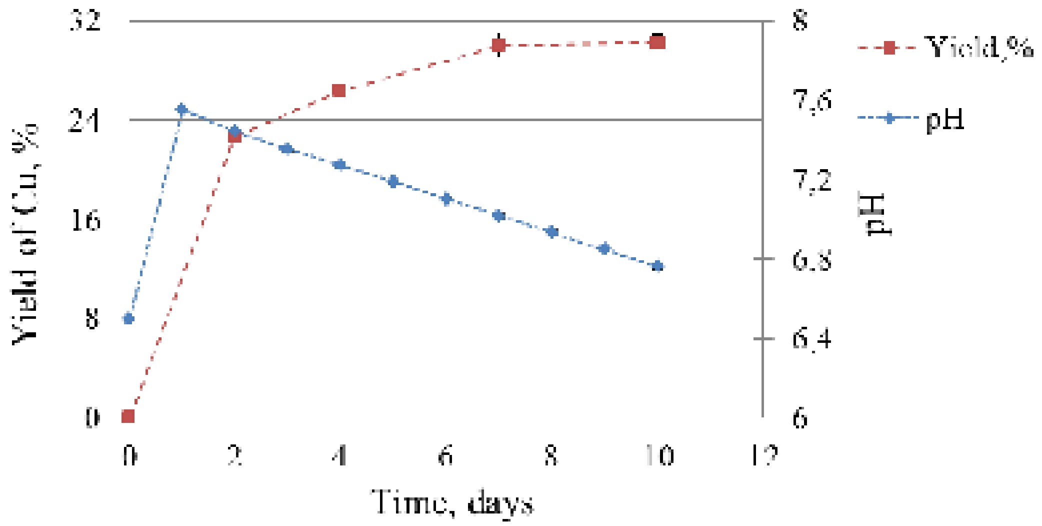


Figure 13 Leaching kinetics of Mansfeld copper shale (deslimed) by PGA

The leaching was performed in Erlenmeyer flasks (refer 2.6.1) at 27°C. Samples were taken at 2nd, 4th, 7th and 10th day and pre-treated as previously described (refer 2.6.2) in order to determine the yield of copper in Mansfeld Copper shale, deslimed hydrocyclone product (-90+50µm) in the cell-free supernatant of *Bacillus licheniformis* with PGA concentration of 12 g/l. The blue line shows pH change over 10 days and the red line copper yield.

Figure 13 illustrates the leaching kinetics of copper from Mansfeld Kupferschiefer ore over a 10 day period and the pH alteration of the leaching solution. The highest Cu yield among experiments with PGA, was reached with the following conditions:

Parameter	Values
Pulp density	10 %
Temperature	37 °C
pH	6.5

After beginning the experiment, the yield steeply increased to 22.76% in two days, and showed further growth on Day four, with the yield of 26.4%. On Day seven, the yield went up to 30%, with a yield plateau occurring until the Day ten, where the yield was 30.26%.

In Figure 13, the second line (blue) demonstrates the pH change during the experiment. The initial pH of the culture broth was 6.5. On the Day two, there was a dramatic jump of the pH to 7.45. A steady decrease was experienced to a pH of 6.77 on Day 10.

3.1.2 Optimization

The outcome of the model is presumed to enhance the yield of copper under optimized conditions.

Figure 14 demonstrates the response surface model based on the results of the Box-Behnken experimental design.

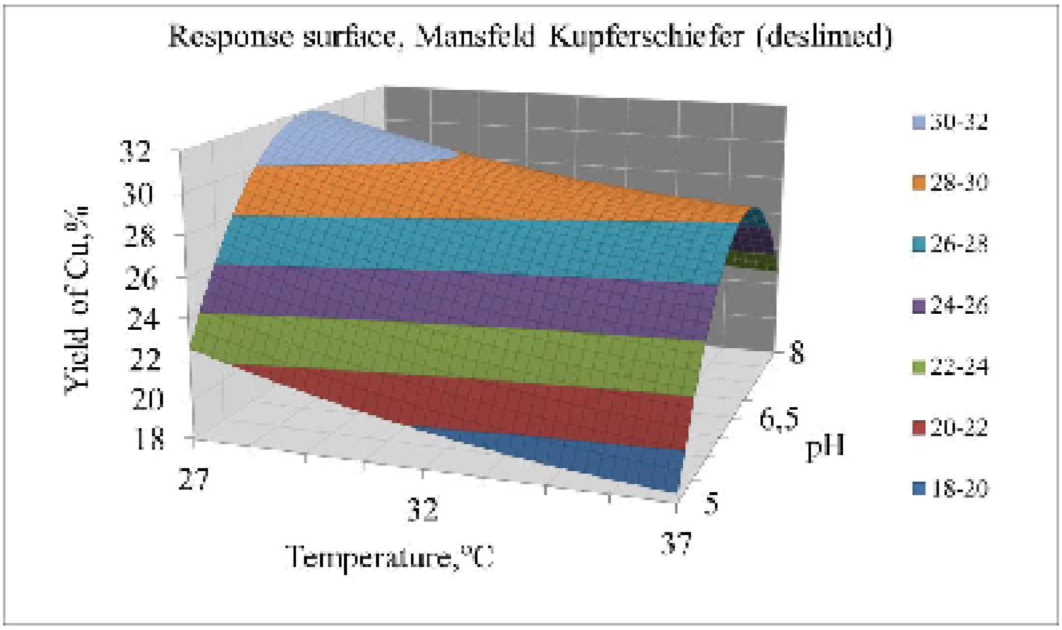


Figure 14 Response surface model for Mansfeld Copper shale (deslimed)

Yield optimization for Mansfeld Copper shale, deslimed hydrocyclone product (-90+50µm) during leaching with cell-free supernatant of *Bacillus licheniformis*. PGA concentration was 12 g/l. The response surface for was calculated based on Box-Behnken design of experiment results (refer 2.6). The statistical significance value was 0.05. Pulp density ratio (w/v) was set at 10 % to show the influence of pH and temperature change on Cu yield.

The pulp density was determined to be optimal when the weight per volume ratio is 10% and is fixed at this value in the model design. The variation of temperature within the range of 27°C-37°C and pH between 5-8, gives a copper yield with respect to a statistical model.

According to the obtained results, the maximal yield can be reached under the following conditions:

Parameter	Optimized value
Pulp density	10 %
Temperature	27 °C
pH	6.68
Yield	31.9

3.1.3 Leaching with stepwise change of supernatant

In order to examine the influence of pretreatment with biologically produced organic acids and ammonium before leaching with PGA experiment with a stepwise change of leaching supernatant was implemented.

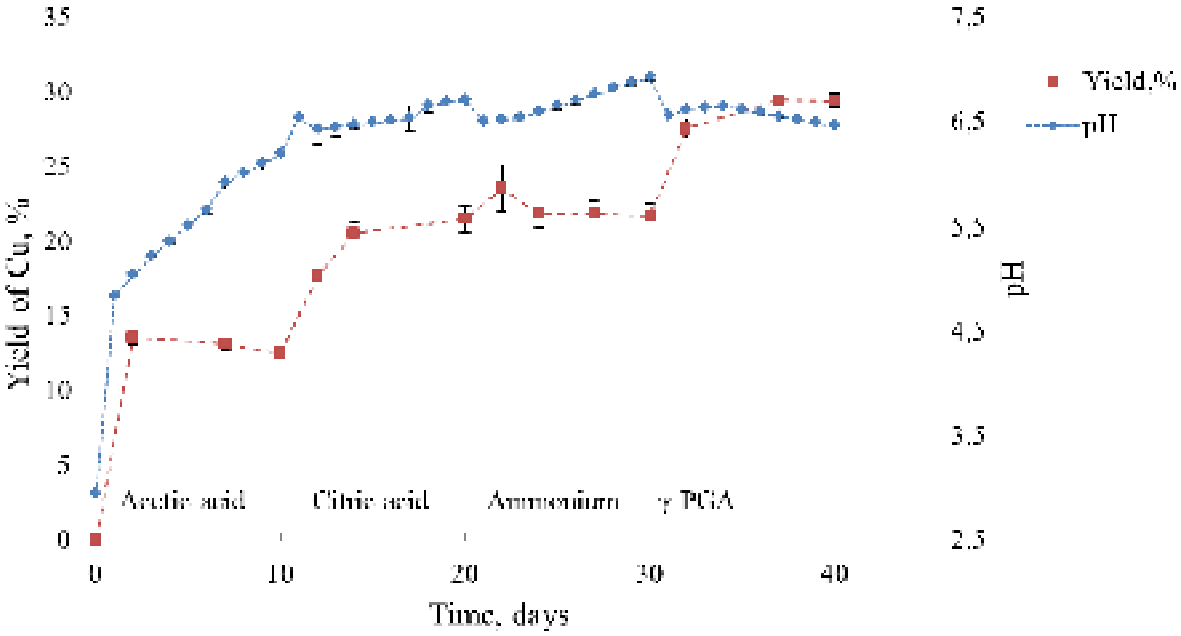


Figure 15 Leaching kinetics of Mansfeld Copper shale (deslimed) with stepwise change of supernatant

Mansfeld Copper shale, deslimed hydrocyclone product (-90+50µm) leaching with a stepwise change of cell-free supernatant containing defined amount of biologically produced leaching agents such as acetic acid by Kombucha-culture, citric acid (6.5 g/l) by *Yarrowia lipolytica*, ammonium (0.5-0.6 g/l) by *Sporosarcina ureae* and PGA by *Bacillus licheniformis*. The blue line shows pH change over 40 days.

Experiment run in an overhead mixer with pulp density = 15 % and lab temperature.

The line Figure 15 illustrates the leaching kinetics of Cu from Mansfeld copper shale Ore over 40 days, and the pH alteration of the leaching solution. The experiment was run with the sample duplicate. Thus, the points are the mean value and the error bars at every measurement point show the standard deviation of a particular value.

In Figure 15, the time axis indicates that the main leaching compounds were utilized in the leaching experiment within a certain time span. During the first 10 days of leaching experiment, the Kombucha culture broth was applied. This resulted in the peak of copper yield on the second day, with the yield of 13.6%. A slight decrease was experienced on Day 7 and a minimum value was seen on the Day 10 with the yield of 12.5%.

After PLS was removed, Mansfeld copper shale ore was washed out with distilled water and mixed with cell-free culture broth of *Yarrowia lipolytica,* which contains 6.5 g/l of citric acid. Following this addition, the experiment was run for ten more days (Days 10 - 20). The yield is cumulative and shows the sum of yields on the last day of leaching with acetic acid (Day 10) and the yield caused by leaching with citric acid on second, fourth, seventh and tenth days. The yield went up significantly and on Day 4, reaching a copper recovery rate of 20.6%. Then slight increase was observed of 21.5% on Day 10.

After ten days of leaching with citric acid, the same PLS removal, and washing procedures were applied, and the culture broth of *Sporosarcina ureae* was added with the ammonium concentration of 0.5-0.6 g/l. The experiment was run for another 10 days (Days 20-30). The yield is cumulative and shows the sum of yields at the last day of leaching with culture broth of *Yarrowia lipolytica* (day 20) and the yield caused by leaching with ammonium on second, fourth, seventh and tenth days.
The yield rose to 21.9% on Day 22 and at the Day 30 experiment shows an overall yield of 21.7% after 10 days of leaching with ammonium.

The culture broth of *Bacillus licheniformis* has a PGA content of 12-13 g/l and was used on Days 30-40 of the leaching experiment.
The copper yield dramatically went up on the second day of leaching with PGA reaching 26.7% and then increased to 29.4% on Day 40.

During the experiment, the pH rapidly increased from 2.94 to 6.2 on Day 10. This is due to the high neutralization potential of carbonates in the ore and tended to be within the limit of 6.3-7.2 varying slightly over next 30 days.

3.1.4 Mineralogical analyze of copper mineral

A number of copper minerals in Mansfeld copper shale ore are less than 1% that interfere precise evaluation of copper minerals content. Due to this fact, minerals distribution not shown, but instead, analyzed ore surface is demonstrated to observe qualitative minerals distribution.

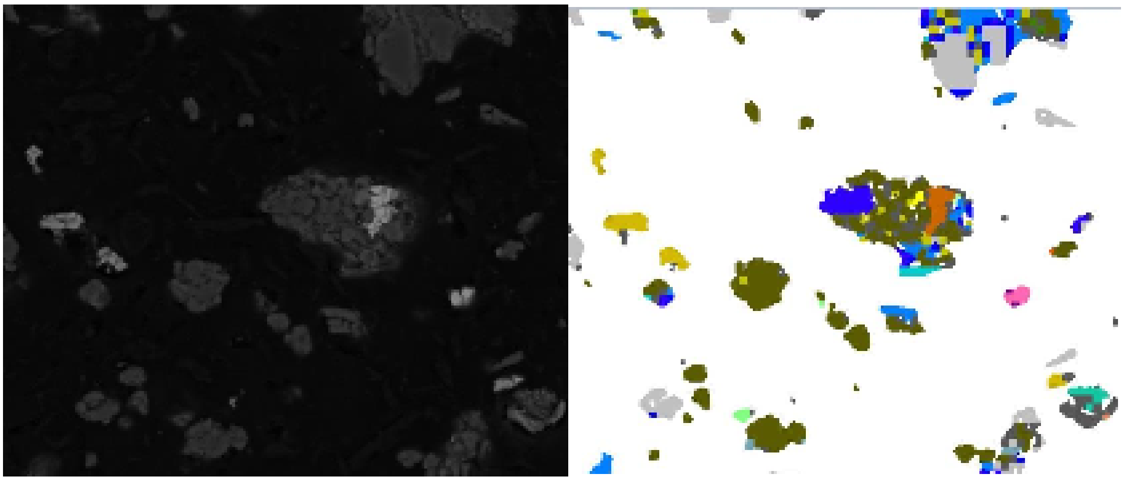


Figure 16 a,b Scanning electron microscopy of leached Mansfeld copper shale (deslimed)

Backscattered electron image (left) of a Mansfeld copper shale containing copper minerals. Bright are sulfide minerals, medium gray carbonate and silicate minerals, dark gray is solid organic matter. It is apparent that the latter has very similar BSE brightness to the epoxy resin surrounding the particle. False-coloured MLA image (right) of the same particle illustrates the presence of the sulfide minerals (in orange, pink and brown color).

Figure 16Figure 16a illustrates Backscattered electron image of the mineral surface and on the Figure 16b false-coloured MLA image that indicates the minerals by applied color filter.

After leaching for 40 days with various biologically produced metabolites, copper sulfides still present in the ore, but exact distribution between copper minerals was not possible for identification due to detection limits restrictions.

3.2 Mansfeld copper shale, hydrocyclone slime

Gangue content characterizes the ore properties and considered to be an important factor which influences the leaching process.

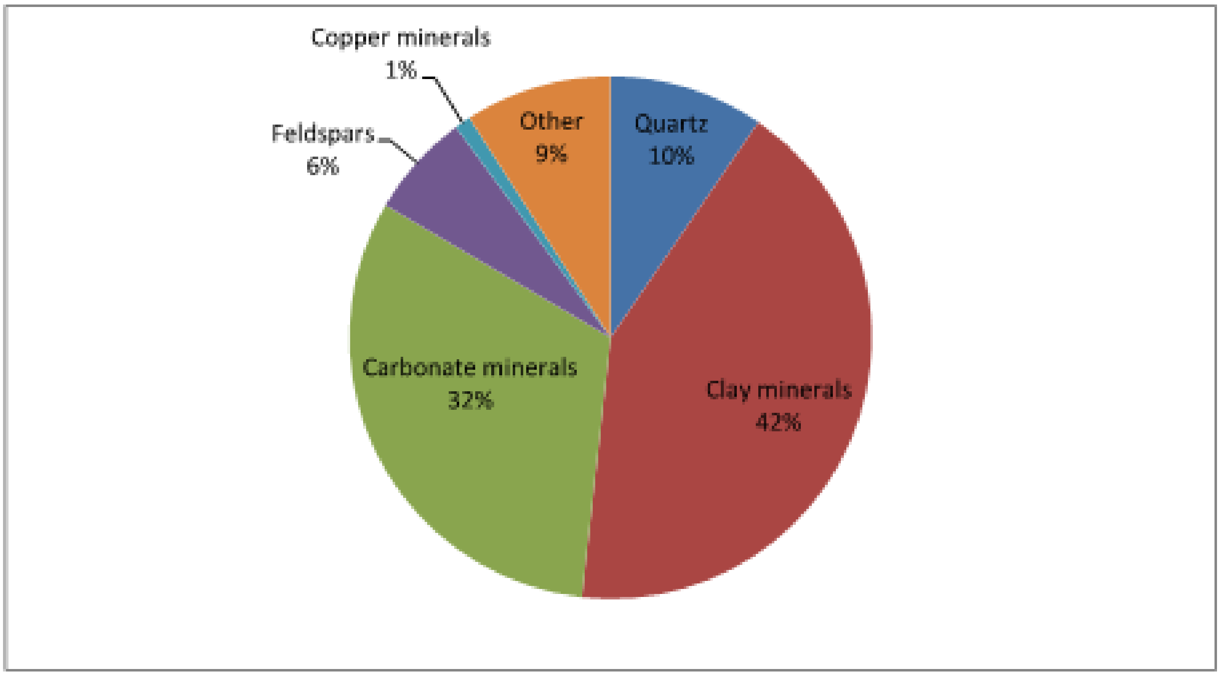


Figure 17 Mineralogical content of the Mansfeld copper shale (slime)

The pie chart shows the main groups of mineral in the Mansfeld copper shale, hydrocyclone slime (-50μm) that describes the gangue content that was measured by XRD.

Figure 17 demonstrates the mineralogical content of the main groups of minerals such as carbonates, clays, quartz, feldspars and metal sulfides measured by XRD for Mansfeld copper shale (hydrocyclone slime).

The largest group of minerals is clay minerals which account for 42% of the total weight. Carbonate minerals make up one-third of the ore. The minor groups of gangue are quartz and feldspars with the 10% and 6% of the weight respectively. The feed ore contains about 1% of copper ore.

3.1.1 Leaching with PGA

Figure 18 illustrates leaching results with the highest copper yield among the experiments using PGA as a leaching agent for Mansfeld copper shale (hydrocyclone slime).

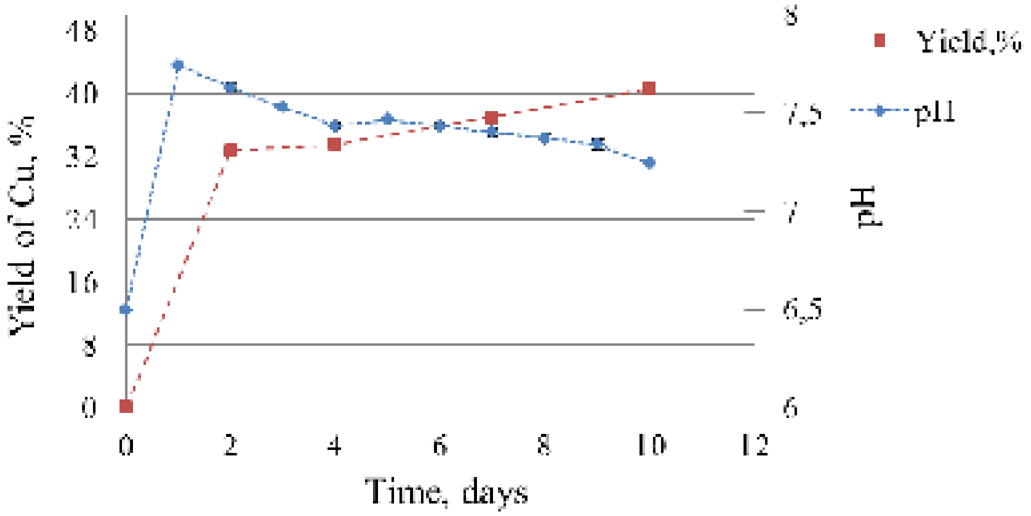


Figure 18 Leaching kinetics of Mansfeld copper shale (slime) by PGA

The leaching was performed in Erlenmeyer flasks (refer 2.6.1) at 27°C. Samples were taken at 2nd, 4th, 7th and 10th day and pretreated as previously described (refer 2.6.2) in order to determine the yield of copper in Mansfeld Copper shale (hydrocyclone slime) (-50µm) in the cell-free supernatant of *Bacillus licheniformis* with PGA concentration of 12 g/l. The blue line shows pH change over 10 days and the red line copper yield.

Figure 18 illustrates the leaching kinetics of copper from Mansfeld copper shale (hydrocyclone slime) ore over a 10 day period and the pH alteration of the leaching solution. The highest Cu yield among experiments with PGA, was reached with the following conditions:

Parameter	Values
Pulp density	10 %
Temperature	27 °C
pH	6.5

After beginning the experiment, the yield steeply increased to 32.78% in two days, and showed further growth on Day four, with the yield of 33.5%. On Day seven, the yield went up to 37% and reached 40.6% on the Day ten.

In Figure 18, the second line (blue) demonstrates the pH change during the experiment. The initial pH of the culture broth was 6.5. On the Day two, there was a dramatic jump of the pH to 7.63. A steady decrease was experienced to a pH of 7.25 on Day 10.

3.2.2 Optimization

The outcome of the model is presumed to enhance the yield of copper under optimized conditions. Figure 19 demonstrates the response surface model based on the results of the Box-Behnken experimental design.

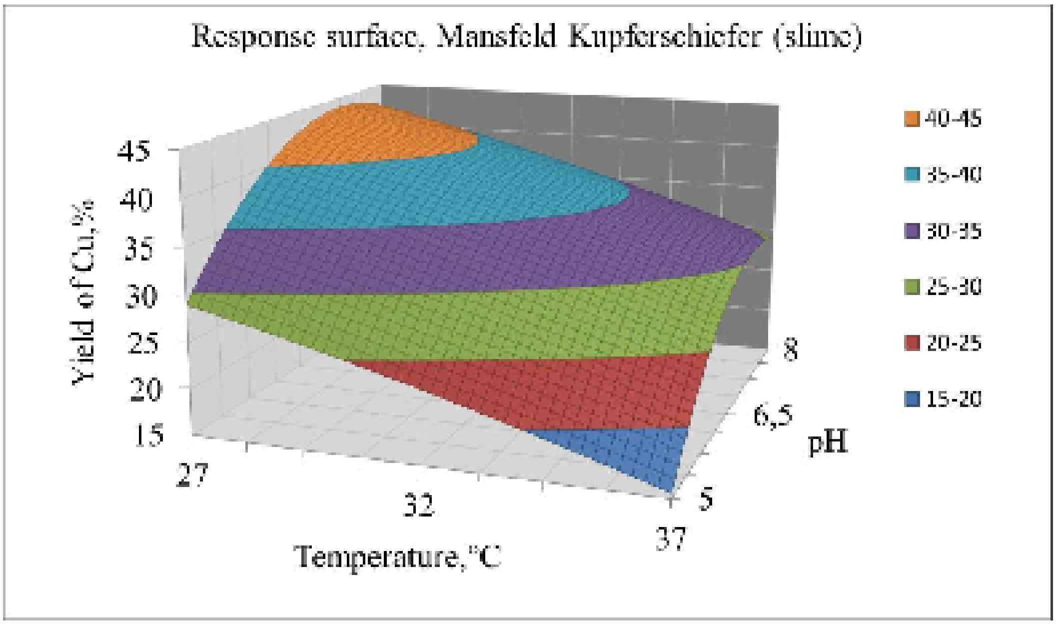


Figure 19 Response surface model for Mansfeld copper shale (slime)

Yield optimization for Mansfeld Copper shale, hydrocyclone slime (-50µm) during leaching with cell-free supernatant of *Bacillus licheniformis* with PGA concentration of 12 g/l. The response surface for was calculated based on Box-Behnken design of experiment results (refer 2.6). The statistical significance value was 0,0062. Pulp density ratio (w/v) was set at 10 % to show the influence of pH and temperature change on Cu yield.

The pulp density was determined to be optimal when the weight per volume ratio is 10% and is fixed at this value in the model design. The variation of temperature within the range of 27°C-37°C and pH between 5-8, gives a copper yield with respect to a statistical model. According to the obtained results, the maximal yield can be reached under the following conditions:

Parameter	Optimized value
Pulp density	10 %
Temperature	27 °C
pH	7.27
Yield	43.9

3.2.3 Leaching with stepwise change of supernatant

In order to examine the influence of pretreatment with biologically produced organic acids and ammonium before leaching with PGA experiment with a stepwise change of leaching supernatant was implemented.

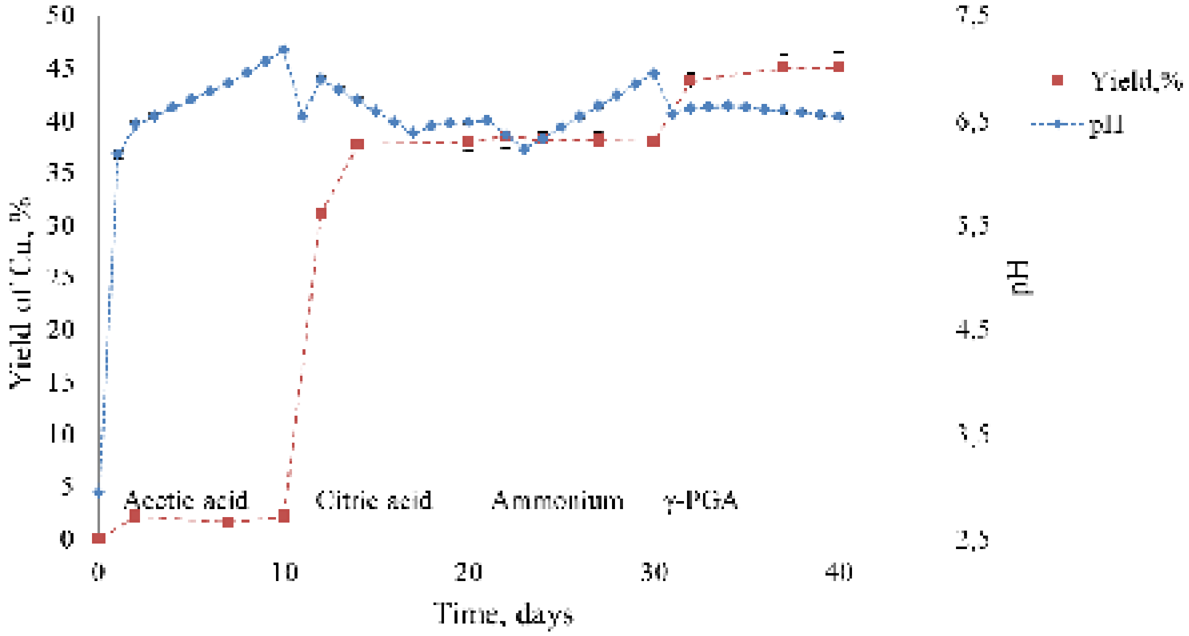


Figure 20 Leaching kinetics of Mansfeld Copper shale (slime) with stepwise change of supernatant

Mansfeld Copper shale, hydrocyclone slime (-50µm) leaching with a stepwise change of cell-free supernatant containing defined amount of biologically produced leaching agents such as acetic acid by Kombucha-culture, citric acid (6.5 g/l) by *Yarrowia lipolytica*, ammonium (0.5-0.6 g/l) by *Sporosarcina ureae* and PGA by *Bacillus licheniformis.* The blue line shows pH change over 40 days.

Experiment run in an overhead mixer with pulp density = 15 % and lab temperature.

The line Figure 20 illustrates the leaching kinetics of Cu from Mansfeld Kupferschiefer Ore over 40 days, and the pH alteration of the leaching solution. The experiment was run with the sample duplicate. Thus, the points are the mean value and the error bars at every measurement point show the standard deviation of a particular value.

In Figure 20, the time axis indicates that the main leaching compounds were utilized in the leaching experiment within a certain time span. During the first 10 days of leaching experiment, the Kombucha culture broth was applied. This resulted in the copper yield on the second day of 2.2%. A slight decrease was experienced on Day 7 and on the Day 10 the yield was 2.15%.

After PLS was removed, Mansfeld copper shale ore was washed out with distilled water and mixed with cell-free culture broth of *Yarrowia lipolytica,* which contains 6.5 g/l of citric acid. Following this addition, the experiment was run for ten more days (Days 10 - 20). The yield is cumulative and shows the sum of yields on the last day of leaching with acetic acid (Day 10) and the yield caused by leaching with citric acid on second, fourth, seventh and tenth days.

The yield went up significantly and on Day 4, reaching a copper recovery rate of 37.9%. Then slight increase was observed of 21.5% on Day 10.

After ten days of leaching with citric acid, the same PLS removal and washing procedures were applied, and the culture broth of *Sporosarcina ureae* was added with the ammonium concentration of 0.5-0.6 g/l. The experiment was run for another 10 days (Days 20-30). The yield is cumulative and shows the sum of yields at the last day of leaching with culture broth of *Yarrowia lipolytica* (day 20) and the yield caused by leaching with ammonium on second, fourth, seventh and tenth days.

The yield slightly rose to 38.5% on Day 22 and at the Day 30 experiment shows an overall yield of 38% after 10 days of leaching with ammonium.

The culture broth of *Bacillus licheniformis* has a PGA content of 12-13 g/l and was used on Days 30-40 of the leaching experiment.

The copper yield dramatically went up on the second day of leaching with PGA reaching 43.8% and then increased to 45.1% on Day 40.

During the experiment, the pH rapidly increased from 2.94 to 6.46 on Day 2. This is due to the high neutralization potential of carbonates in the ore and tended to be within the limit of 6.2-7.15 varying slightly over next 30 days.

3.2.4 Mineralogical analyze of copper mineral

A number of copper minerals in Mansfeld copper shale ore are less than 1% that interfere precise evaluation of copper minerals content. Due to this fact, minerals distribution not shown, but instead, analyzed ore surface is demonstrated to observe qualitative minerals distribution.

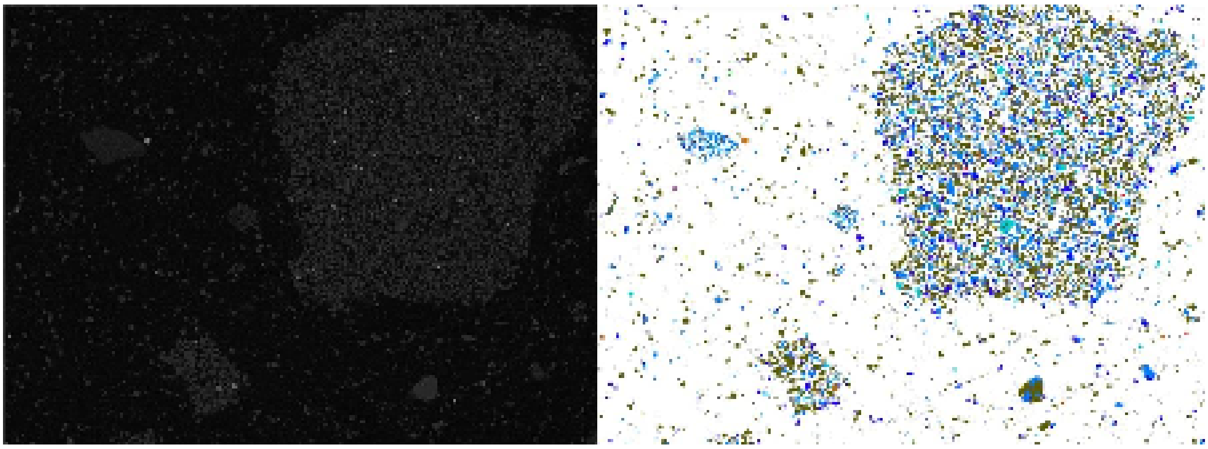


Figure 21 a,b Scanning electron microscopy of leached Mansfeld copper shale (slime)

Backscattered electron image (left) of a Mansfeld copper shale (slime) containing copper minerals. Bright are sulfide minerals, medium gray carbonate and silicate minerals, dark gray is solid organic matter. It is apparent that the latter has very similar BSE brightness to the epoxy resin surrounding the particle. False-coloured MLA image (right) of the same particle illustrates the presence of the sulfide minerals (in orange, pink and brown color)

Figure 21 a illustrates Backscattered electron image of the mineral surface and on the Figure 21b false-coloured MLA image that indicates the minerals by applied color filter.
After leaching for 40 days with various biologically produced metabolites, copper sulfides still present in the ore, but exact distribution between copper minerals was not possible for identification due to detection limits restrictions.

3.3 Sangerhausen copper shale

Gangue content characterizes the ore properties and considered to be an important factor which influences the leaching process.

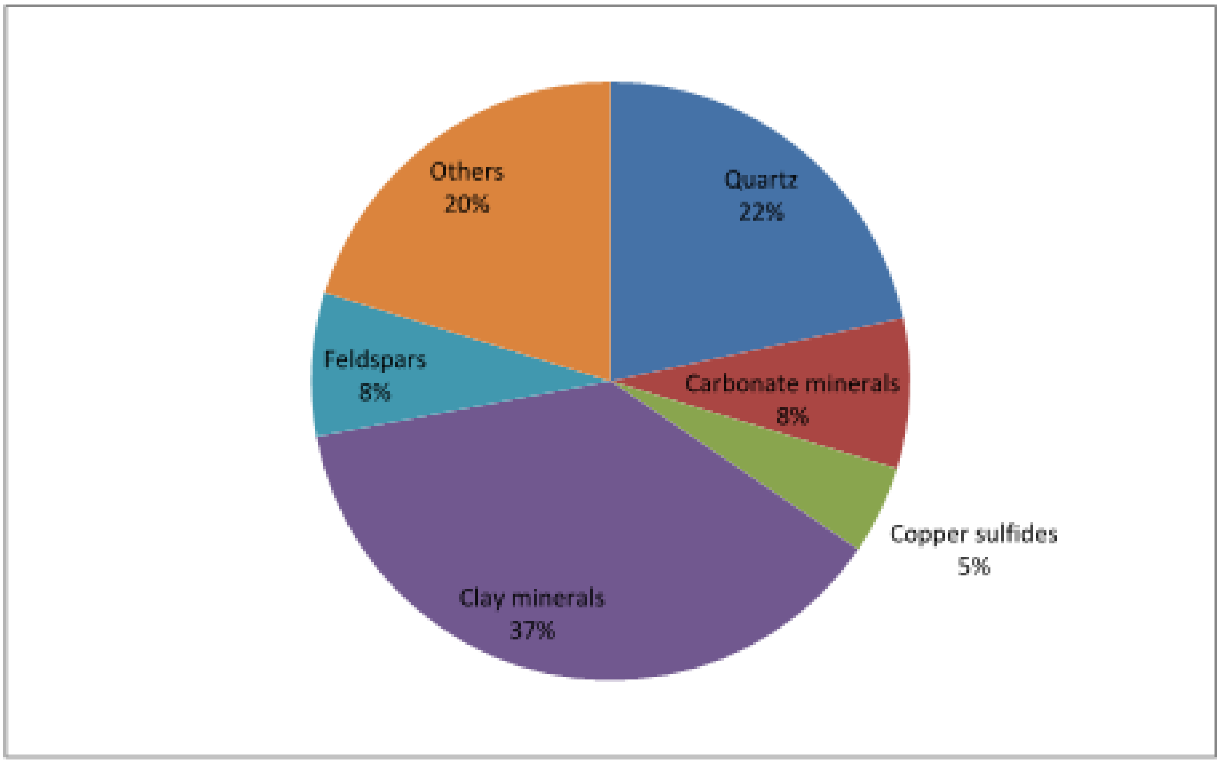


Figure 22 Mineralogical content of the Sangerhausen copper shale

The pie chart shows the main groups of mineral in the Sangerhausen Copper shale, (-90µm) that describes the gangue content that was measured by XRD

Figure 22 demonstrates the mineralogical content of the main groups of minerals such as carbonates, clays, quartz, feldspars and metal sulfides measured by XRD for Sangerhausen copper shale.

The largest group of minerals is clay minerals which account for 37% of the total weight. Quatz makes up almost a quarter of the ore. The minor groups of gangue are carbonates and feldspars with the 8% of the weight each. The feed ore contains 5% of copper sulfides.

3.3.1 Leaching with PGA

Figure 23 illustrates leaching results with the highest copper yield among the experiments using PGA as a leaching agent for Sangerhausen copper shale.

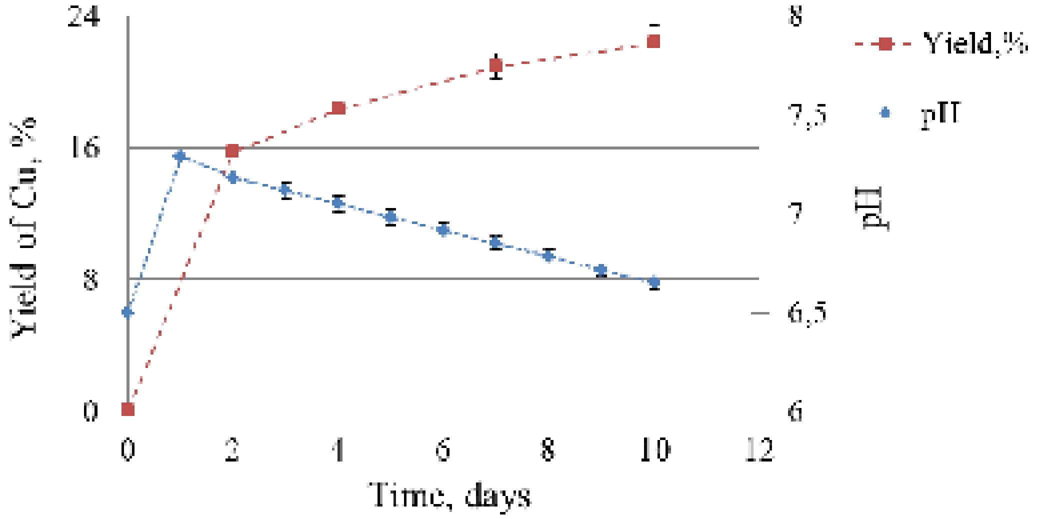


Figure 23 Leaching kinetics of Sangerhausen copper shale by PGA

The leaching was performed in Erlenmeyer flasks (refer 2.6.1) at 27°C. Samples were taken at 2nd, 4th, 7th and 10th day and pretreated as previously described (refer 2.6.2) in order to determine the yield of copper in Sangerhausen Copper shale (-90µm) in the cell-free supernatant of *Bacillus licheniformis* with PGA concentration of 12 g/l. The blue line shows pH change over 10 days and the red line copper yield.

The Figure 23 illustrates the leaching kinetics of copper from Sangerhausen copper shale ore over a 10 day period and the pH alteration of the leaching solution. The highest Cu yield among experiments with PGA, was reached with the following conditions:

Parameter	Values
Pulp density	10 %
Temperature	37 °C
pH	6.5

After beginning the experiment, the yield steeply increased to 15.7% in two days, and showed further growth on Day four, with the yield of 18.4%. On Day seven, the yield went up to 21%, and then the yield reached 22.4% on Day 10.

In Figure 23, the second line (blue) demonstrates the pH change during the experiment. The initial pH of the culture broth was 6.5. On the Day one, there was a dramatic jump of the pH to 7.3. A steady decrease was experienced to a pH of 6.65 on Day 10.

Due to statistical significance value above the limits of 0.05, the response surface model may not be considered as a reliable and excluded from the results.

3.2.2 Leaching with stepwise change of supernatant

In order to examine the influence of pretreatment with biologically produced organic acids and ammonium before leaching with PGA experiment with a stepwise change of leaching supernatant was implemented.

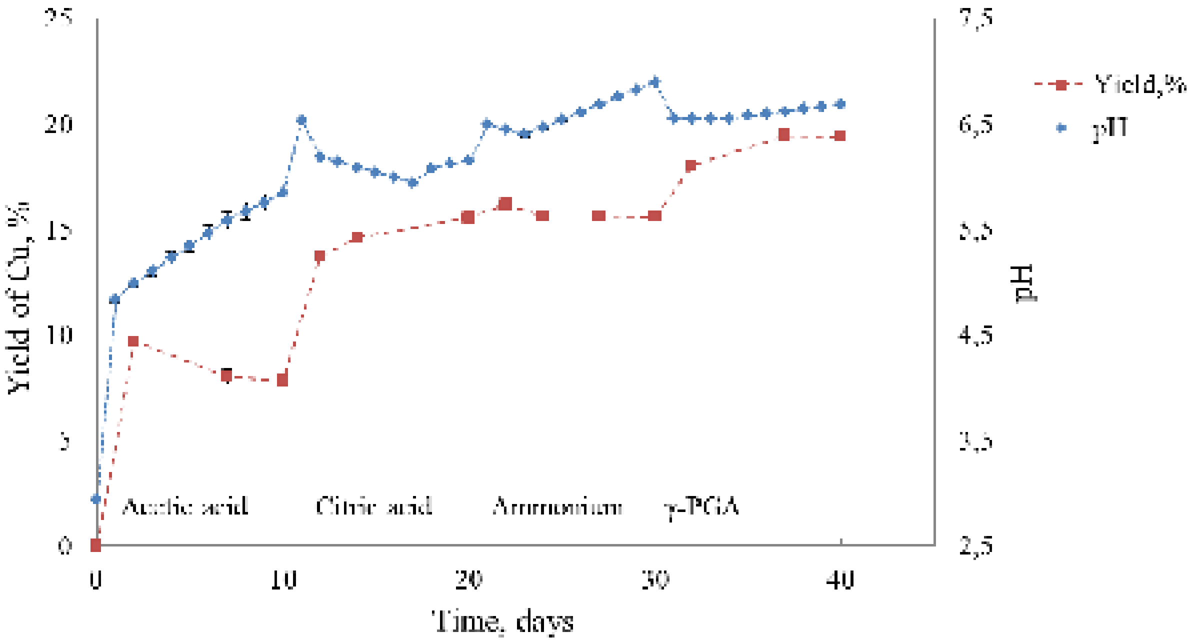


Figure 24 Leaching kinetics of Sangerhausen copper shale with stepwise change of supernatant

Leaching kinetics of Sangerhausen copper shale with stepwise change of supernatant
Leaching with a stepwise change of cell-free supernatant containing defined amount of biologically produced leaching agents such as acetic acid by Kombucha-culture, citric acid (6.5 g/l) by *Yarrowia lipolytica*, ammonium (0.5-0.6 g/l) by *Sporosarcina ureae* and PGA by *Bacillus licheniformis*. The blue line shows pH change over 40 days. Experiment run in an overhead mixer with pulp density = 15 % and lab temperature.

The line Figure 24 illustrates the leaching kinetics of Cu from Sangerhausen copper shale ore over 40 days and the pH alteration of the leaching solution. The experiment was run with the sample duplicate. Thus, the points are the mean value and the error bars at every measurement point show the standard deviation of a particular value.

In Figure 24, the time axis indicates that the main leaching compounds were utilized in the leaching experiment within a certain time span. During the first 10 days of leaching experiment, the Kombucha culture broth was applied. This resulted in the peak of copper yield on the second day, with the yield of 9.7%. A decrease was experienced on Day 7 and a minimum value was seen on the Day 10 with the yield of 7.8%.

After PLS was removed, Mansfeld copper shale ore was washed out with distilled water and mixed with cell-free culture broth of *Yarrowia lipolytica,* which contains 6.5 g/l of citric acid. Following this addition, the experiment was run for ten more days (Days 10 - 20). The yield is cumulative and shows the sum of yields on the last day of leaching with acetic acid (Day 10) and the yield caused by leaching with citric acid on second, fourth, seventh and tenth days. The yield went up significantly and on Day 4, reaching a copper recovery rate of 14.6%. Then slight increase was observed of 15.6% on Day 10.

After ten days of leaching with citric acid, the same PLS removal and washing procedures were applied, and the culture broth of *Sporosarcina ureae* was added with the ammonium concentration of 0.5-0.6 g/l. The experiment was run for another 10 days (Days 20-30). The yield is cumulative and shows the sum of yields at the last day of leaching with culture broth of *Yarrowia lipolytica* (day 20) and the yield caused by leaching with ammonium on second, fourth, seventh and tenth days.
The yield rose to 16.2% on Day 22 and then plateau out until the Day 30 decreasing yield 15.6% after 10 days of leaching with ammonium.

The culture broth of *Bacillus licheniformis* has a PGA content of 12-13 g/l and was used on Days 30-40 of the leaching experiment.
The copper yield went up on the second day of leaching with PGA reaching 18.1% and then reached 19.4% on Day 40.

During the experiment, the pH rapidly increased from 2.94 to 6.54 on Day 10. This is due to the high neutralization potential of carbonates in the ore and tended to be within the limit of 5.8-7.1 varying slightly over next 30 days.

3.3.3 Mineralogical analyze of copper mineral

In order to examine an impact of applied biologically produced leaching agents on mineral content, copper sulfides were measured before and after leaching experiment.

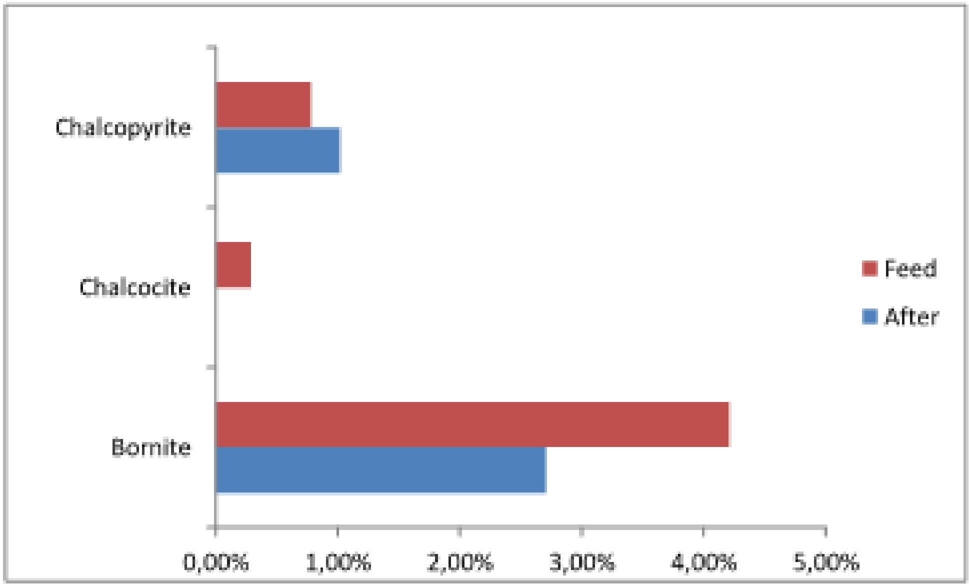


Figure 25 Mineralogical analyses of copper sulfides in Sangerhausen copper shale before and after leaching

Mineralogical analyses of copper sulfides were performed by MLA at 25 keV for feed Sangerhausen copper shale ore and for remaining solid ore sample after leaching with a stepwise change of supernatants (refer 2.6.2). Minerals content are overestimated (refer discussion) but provides the qualitative characteristic of leaching efficiency with respect to copper minerals. Not identified measured area is 15,9% (mostly due to mixed spectra from multiple minerals)

Figure 25 demonstrates the changes in detected copper minerals in feed ore and remaining copper sulfides after leaching. Quantitative distribution of the minerals are not realistic due to overestimation but provides an overview if the mineral became dissolved, remain undissolved or mineral formation took place during the leaching experiment. Due to overestimation, only the trends are interpreted without numerical consideration.

According to MLA results, chalcocite was completely dissolved, about 40% of bornite was dissolved. After leaching the weight of chalcopyrite increased by approximately 20%, that could be induced either by additional chalcopyrite formation or due to the challenges in mineral sample surface identification.

3.4 Rudna Sandstone

Gangue content characterizes the ore properties and considered to be an important factor which influences the leaching process.

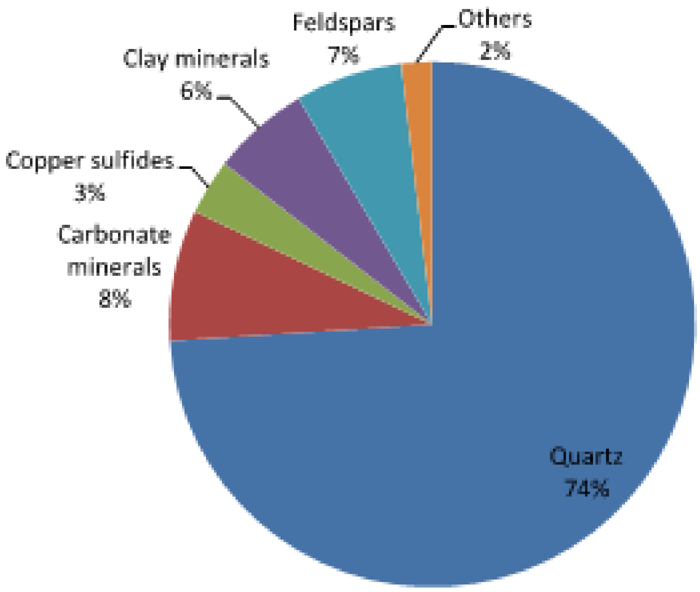


Figure 26 Mineralogical content of the (deslimed)

The pie chart shows the main groups of mineral in Rudna sandstone (-90µm) that describes the gangue content that was measured by XRD.

The chart Figure 26 demonstrates the mineralogical content of the main groups of minerals such as carbonates, clays, quartz, feldspars and metal sulfides measured by XRD for Rudna sandstone.

The largest group of minerals is quartz which makes up almost three-quarter of the total weight. The minor groups of gangue are carbonates, feldspars and clay minerals which distributed almost equal and account for 8%, 7% and 6% of the weight respectively. The feed ore contains 3% of copper ore.

3.4.1 Leaching with PGA

Figure 27 illustrates leaching results with the highest copper yield among the experiments using PGA as a leaching agent for Rudna sandstone ore.

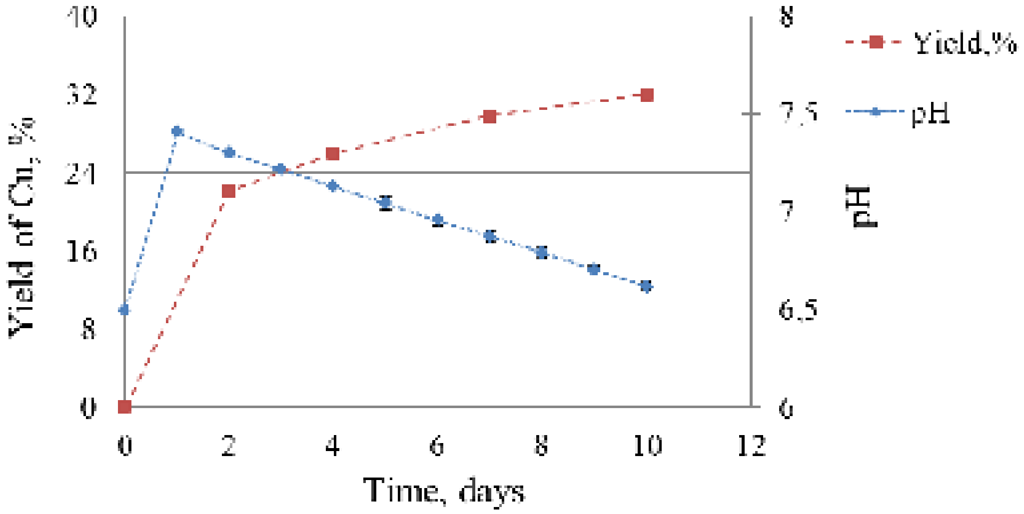


Figure 27 Leaching kinetics of Rudna sandstone by PGA

The leaching was performed in Erlenmeyer flasks (refer 2.6.1) at 27°C. Samples were taken at 2nd, 4th, 7th and 10th day and pretreated as previously described (refer 2.6.2) in order to determine the yield of copper in Rudna sandstone (-90µm) in the cell-free supernatant of *Bacillus licheniformis* with PGA concentration of 12 g/l. The blue line shows pH change over 10 days and the red line copper yield.

The line Figure 26 illustrates the leaching kinetics of copper from Rudna sandstone ore over a 10 day period and the pH alteration of the leaching solution. The highest Cu yield among experiments with PGA, was reached with the following conditions:

Parameter	Values
Pulp density	10 %
Temperature	37 °C
pH	6.5

After beginning the experiment, the yield steeply increased to 22.1% in two days, and showed further growth on Day four, with the yield of 26%. On Day seven, the yield went up to 29.9%, with a yield plateau occurring until the Day ten, where the yield was 32%.

In Figure 27, the second line (blue) demonstrates the pH change during the experiment. The initial pH of the culture broth was 6.5. On the Day one, there was a dramatic jump of the pH to 7.41. A steady decrease was experienced to a pH of 6.62 on Day 10.

Due to statistical significance value above the limits of 0.05, the response surface model may not be considered as a reliable and excluded from the results.

3.4.2 Leaching with stepwise change of supernatant

In order to examine the influence of pretreatment with biologically produced organic acids and ammonium before leaching with PGA experiment with a stepwise change of leaching supernatant was implemented.

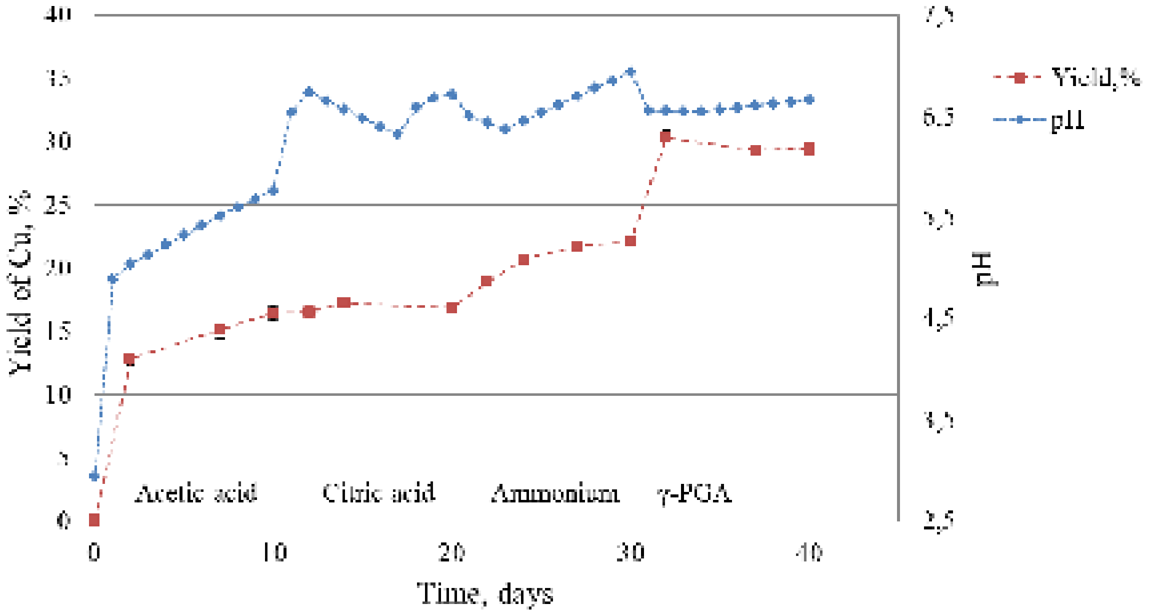


Figure 28 Leaching kinetics of Rudna sandstone with stepwise change of supernatant

Rudna sandstone (-90µm) leaching with a stepwise change of cell-free supernatant containing defined amount of biologically produced leaching agents such as acetic acid by Kombucha-culture, citric acid (6.5 g/l) by *Yarrowia lipolytica*, ammonium (0.5-0.6 g/l) by *Sporosarcina ureae* and PGA by *Bacillus licheniformis*. The blue line shows pH change over 40 days. Experiment run in an overhead mixer with pulp density = 15 % and lab temperature.

The line Figure 28 illustrates the leaching kinetics of Cu from Rudna sandstone ore over 40 days, and the pH alteration of the leaching solution. The experiment was run with the sample duplicate. Thus, the points are the mean value and the error bars at every measurement point show the standard deviation of a particular value.

In Figure 28, the time axis indicates that the main leaching compounds were utilized in the leaching experiment within a certain time span. During the first 10 days of leaching experiment, the Kombucha culture broth was applied. This resulted in the peak of copper yield on the second day, with the yield of 12.8%. A slight increase was experienced on Day 7 and a maximum value was seen on the Day 10 with the yield of 16.5%.

After PLS was removed, Mansfeld copper shale ore was washed out with distilled water and mixed with cell-free culture broth of *Yarrowia lipolytica,* which contains 6.5 g/l of citric acid. Following this addition, the experiment was run for ten more days (Days 10 - 20). The yield is cumulative and shows the sum of yields on the last day of leaching with acetic acid (Day 10) and the yield caused by leaching with citric acid on second, fourth, seventh and tenth days. The yield slightly went up and on Day 4, reaching a copper recovery rate of 17.2%. Then slight decrease was observed in 16.8% on Day 10.

After ten days of leaching with citric acid, the same PLS removal and washing procedures were applied, and the culture broth of *Sporosarcina ureae* was added with the ammonium concentration of 0.5-0.6 g/l. The experiment was run for another 10 days (Days 20-30). The yield is cumulative and shows the sum of yields at the last day of leaching with culture broth of *Yarrowia lipolytica* (day 20) and the yield caused by leaching with ammonium on second, fourth, seventh and tenth days.
The yield rose to 18.9% on Day 22 and at the Day 30 experiment shows an overall yield of 22.1% after 10 days of leaching with ammonium.

The culture broth of *Bacillus licheniformis* has a PGA content of 12-13 g/l and was used on Days 30-40 of leaching experiment.

The copper yield dramatically went up on the second day of leaching with PGA reaching 30.3% and then decreased to 29.4% on Day 40.

During the experiment, the pH rapidly increased from 2.94 to 6.54 on Day 10. This is due to the high neutralization potential of carbonates in the ore and tended to be within the limit of 6.3-6.9 varying slightly over next 30 days.

3.4.3 Mineralogical analyze of copper mineral

In order to examine an impact of applied biologically produced leaching agents on mineral content, copper sulfides were measured before and after leaching experiment.

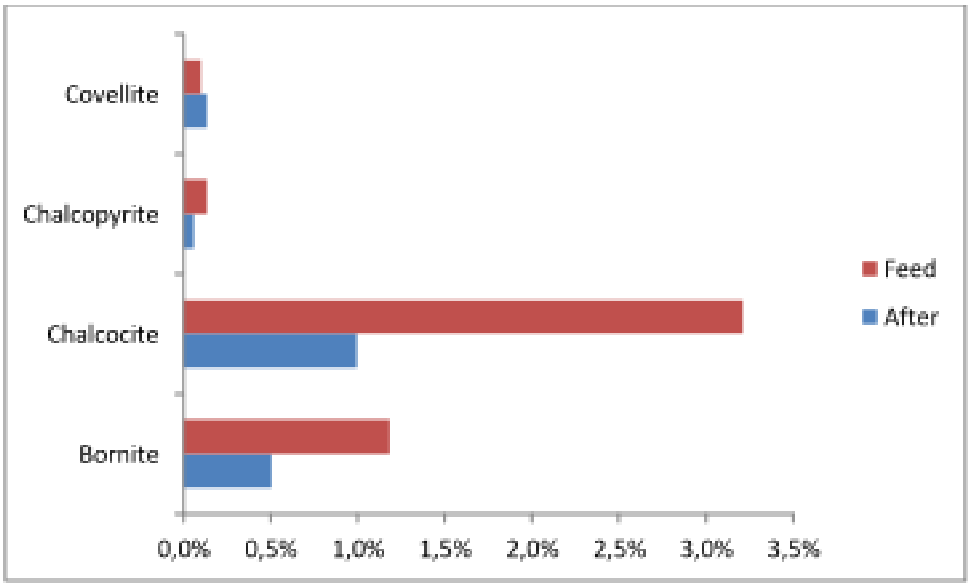


Figure 29 Mineralogical analyses of copper sulfides in Rudna sandstone before and after leaching

Mineralogical analyses of copper sulfides were performed by MLA at 25 keV for feed Rudna sandstone ore and for remaining solid ore sample after leaching with a stepwise change of supernatants (refer 2.6.2) . Minerals content are overestimated (refer discussion) but provides the qualitative characteristic of leaching efficiency with respect to copper minerals. Not identified measured area is 1.72% (mostly due to mixed spectra from multiple minerals).

Figure 29 demonstrates the changes in detected copper minerals in feed ore and remaining copper sulfides after leaching. Quantitative distributions of the minerals are not realistic due to overestimation, but provides an overview whether the mineral became dissolved or remain undissolved. Due to overestimation, only the trends are interpreted without numerical consideration.

According to MLA results, chalcocite which represents more than half of copper minerals was reduced by three-quarter, a noticeable part of bornite was dissolved that account for about 60%. After leaching the weight of chalcopyrite which is minor mineral in Rudna sandstone, decreased by about 80% meanwhile small portion of covellite formed.

3.5 Rudna shale

Gangue content characterizes the ore properties and considered to be an important factor which influences the leaching process.

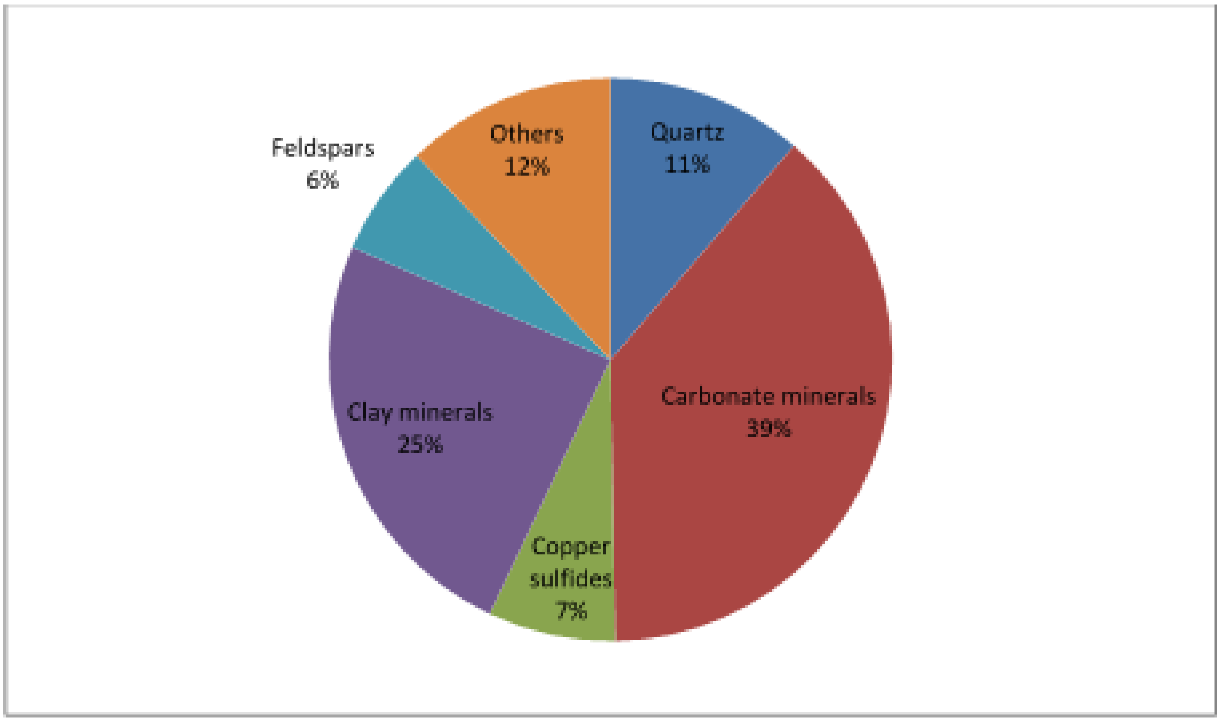


Figure 30 Mineralogical content of the Rudna copper shale

The pie chart shows the main groups of mineral in the Rudna copper shale (-90μm) that describes the gangue content that was measured by XRD.

The chart Figure 30 demonstrates the mineralogical content of the main groups of minerals such as carbonates, clays, quartz, feldspars and metal sulfides measured by XRD for Rudna copper shale ore.

The largest group of minerals is carbonate minerals which account for 39% of the total weight. Clay minerals make up a quarter of the ore. The minor groups of gangue are quartz and feldspars with the 11% and 6% of the weight respectively. The feed ore contains about 7% of copper ore.

3.5.1 Leaching with PGA

Figure 3.2 Figure 31 illustrates leaching results with the highest copper yield among the experiments using PGA as a leaching agent for Rudna copper shale.

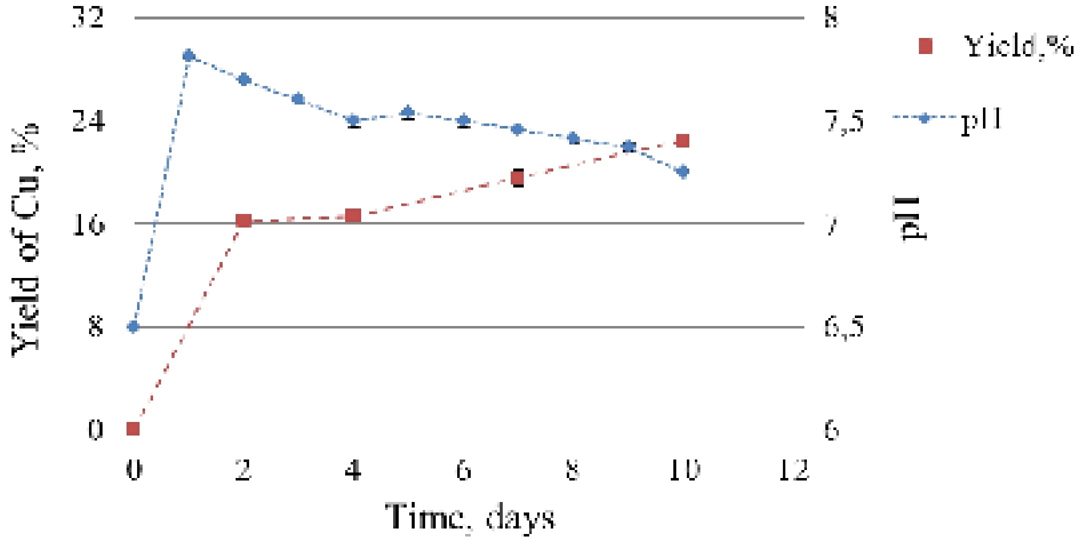


Figure 31 Leaching kinetics of Rudna copper shale by PGA

The leaching was performed in Erlenmeyer flasks (refer 2.6.1) at 27°C. Samples were taken at 2nd, 4th, 7th and 10th day and pretreated as previously described (refer 2.6.2) in order to determine the yield of copper in Rudna copper shale (-90µm) in the cell-free supernatant of *Bacillus licheniformis* with PGA concentration of 12 g/l. The blue line shows pH change over 10 days and the red line copper yield.

The line Figure 31 illustrates the leaching kinetics of copper from Rudna copper shale ore over a 10 day period and the pH alteration of the leaching solution. The highest Cu yield among experiments with PGA, was reached with the following conditions:

Parameter	Values
Pulp density	10 %
Temperature	27 °C
pH	6.5

After beginning the experiment, the yield steeply increased to 16.2% in two days, and showed slight growth on Day four, with the yield of 16.6%. On Day seven, the yield went up to 19.6% and reached 22.5% on the Day ten.

In Figure 31, the second line (blue) demonstrates the pH change during the experiment. The initial pH of the culture broth was 6.5. On the Day one, there was a dramatic jump of the pH to 7.81. A steady decrease was experienced to a pH of 7.25 on Day 10.

3.5.2 Optimization

The outcome of the model is presumed to enhance the yield of copper under optimized conditions.

Figure 32 demonstrates the response surface model based on the results of the Box-Behnken experimental design.

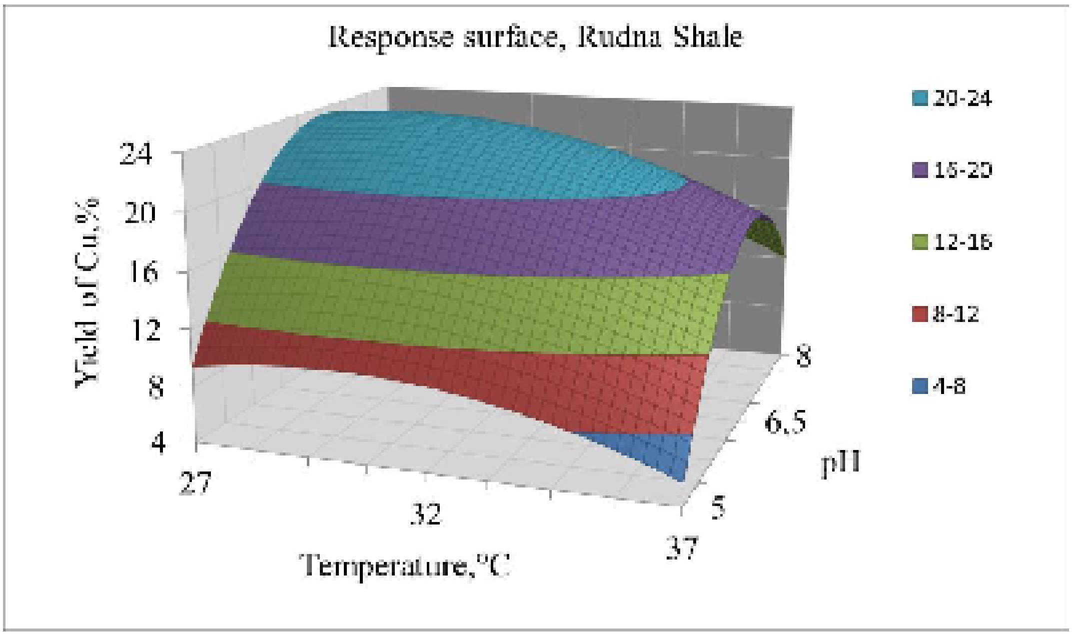


Figure 32 Response surface model for Rudna copper shale

The yield of Cu optimization for Rudna copper shale ore (-90μm) during leaching with cell-free supernatant of *Bacillus licheniformis* with PGA concentration of 12 g/l. The response surface for was calculated based on Box-Behnken design of experiment results (refer 2.6). The statistical significance value was 0,041. Pulp density ratio (w/v) was set at 10 % to show the influence of pH and temperature change on Cu yield.

The pulp density was determined to be optimal when the weight per volume ratio is 10% and is fixed at this value in the model design. The variation of temperature within the range of 27°C-37°C and pH between 5-8, gives a copper yield with respect to a statistical model.

According to the obtained results, the maximal yield can be reached under the following conditions:

Parameter	Optimized value
Pulp density	10 %
Temperature	27 °C
pH	7.27
Yield	23.9

3.5.3 Leaching with stepwise change of supernatant

In order to examine the influence of pretreatment with biologically produced organic acids and ammonium before leaching with PGA experiment with a stepwise change of leaching supernatant was implemented.

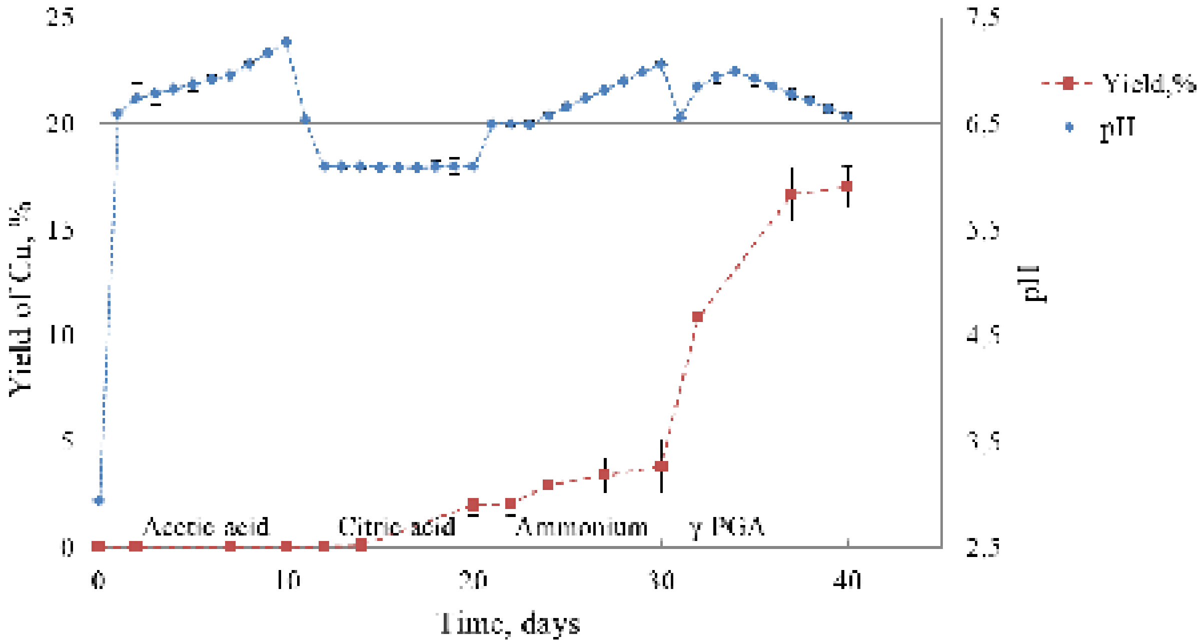


Figure 33 Leaching kinetics of Rudna sandstone with stepwise change of supernatant

Leaching kinetics of Rudna copper shale with stepwise change of supernatant

Rudna copper shale (-90µm) leaching with a stepwise change of cell-free supernatant containing defined amount of biologically produced leaching agents such as acetic acid by Kombucha-culture, citric acid (6.5 g/l) by *Yarrowia lipolytica*, ammonium (0.5-0.6 g/l) by *Sporosarcina ureae* and PGA by *Bacillus licheniformis*. The blue line shows pH change over 40 days. Experiment run in an overhead mixer with pulp density = 15 % and lab temperature.

The line Figure 33 illustrates the leaching kinetics of Cu from Rudna copper shale ore over 40 days and the pH alteration of the leaching solution. The experiment was run with the sample duplicate. Thus, the points are the mean value and the error bars at every measurement point show the standard deviation of a particular value.

In Figure 33, the time axis indicates that the main leaching compounds were utilized in the leaching experiment within a certain time span. During the first 10 days of leaching experiment, the Kombucha culture broth was applied. There was no copper extraction over the whole experiment.

After PLS was removed, Mansfeld copper shale ore was washed out with distilled water and mixed with cell-free culture broth of *Yarrowia lipolytica,* which contains 6.5 g/l of citric acid. Following this addition, the experiment was run for ten more days (Days 10 - 20). The yield is cumulative and shows the sum of yields on the last day of leaching with acetic acid (Day 10) and the yield caused by leaching with citric acid on second, fourth, seventh and tenth days. The yield bottom out demonstrating no Cu extraction until the Day four. Then slight increase was observed in 2% on Day 10.

After ten days of leaching with citric acid, the same PLS removal and washing procedures were applied, and the culture broth of *Sporosarcina ureae* was added with the ammonium concentration of 0.5-0.6 g/l. The experiment was run for another 10 days (Days 20-30). The yield is cumulative and shows the sum of yields at the last day of leaching with culture broth of *Yarrowia lipolytica* (day 20) and the yield caused by leaching with ammonium on second, fourth, seventh and tenth days.
The yield remained stable on Day 22 and at the Day 30 experiment shows an overall yield of 3.9% after 10 days of leaching with ammonium.

The culture broth of *Bacillus licheniformis* has a PGA content of 12-13 g/l and was used on Days 30-40 of leaching experiment.
The copper yield dramatically went up on the second day of leaching with PGA reaching 10.9% and then gradually increased to 17% on Day 40.

During the experiment, the pH rapidly increased from 2.94 to 7.27 on Day 10. This is due to the high neutralization potential of carbonates in the ore and tended to be within the limit of 6-6.9 varying slightly over next 30 days.

3.5.4 Mineralogical analyze of copper mineral

In order to examine an impact of applied biologically produced leaching agents on mineral content, copper sulfides were measured before and after leaching experiment.

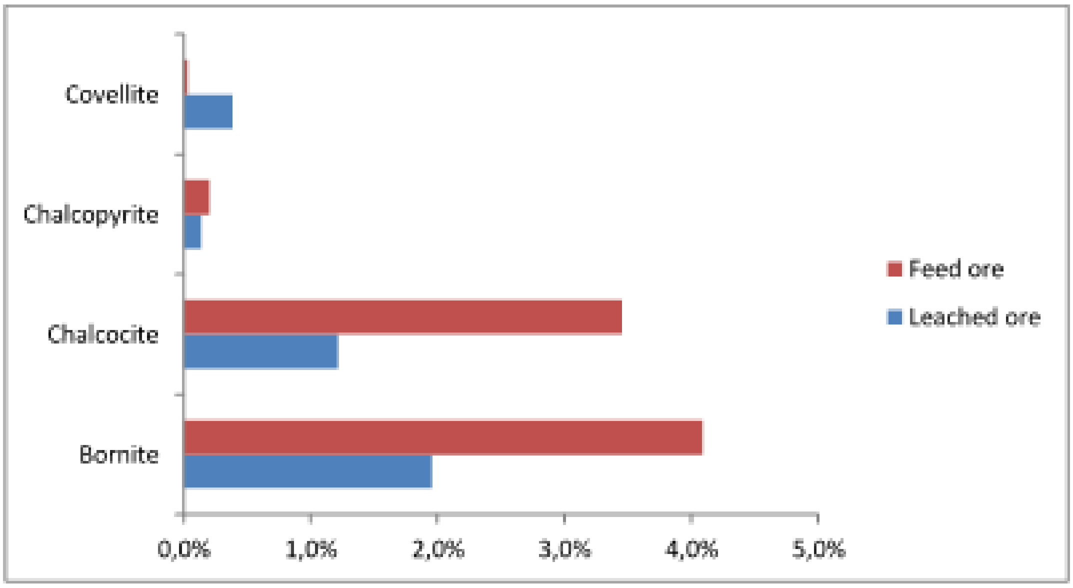


Figure 34 Mineralogical analyses of copper sulfides in Rudna copper shale before and after leaching

Mineralogical analyses of copper sulfides were performed by MLA at 25 keV for feed Rudna copper shale ore and for remaining solid ore sample after leaching with a stepwise change of supernatants (refer 2.6.2). Minerals content are overestimated (refer discussion) but provides the qualitative characteristic of leaching efficiency with respect to copper minerals. Not identified measured area is 19.3% (mostly due to mixed spectra from multiple minerals).

Figure 34 demonstrates the changes in detected copper minerals in feed ore and remaining copper sulfides after leaching. Quantitative distribution of the minerals is not realistic due to overestimation but provides an overview whether the mineral became dissolved or remain undissolved. Due to overestimation, only the trends are interpreted without numerical consideration.

According to MLA results, chalcocite which represents about 40 % of copper minerals was dissolved by 70%, a noticeable part of bornite of about 60% was dissolved. After leaching the weight of chalcopyrite, which is minor mineral in Rudna copper shale, slightly decreased, while a small amount of covellite additionally formed.

3.6 Rudna Carbonate

Gangue content characterizes the ore properties and considered to be an important factor which influences the leaching process.

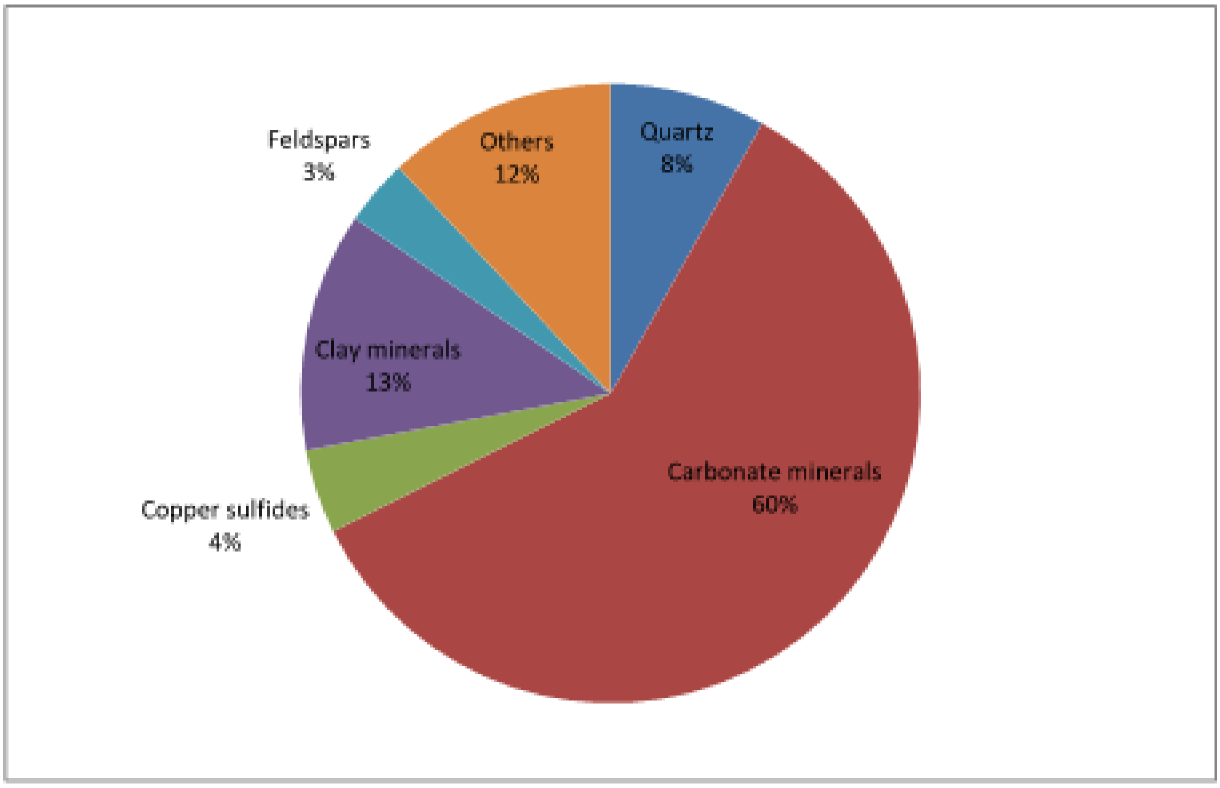


Figure 35 Mineralogical content of the Rudna Carbonate

The pie chart shows the main groups of mineral Rudna Carbonate (-90µm) that describes the gangue content that was measured by XRD.

The chart Figure 35 demonstrates the mineralogical content of the main groups of minerals such as carbonates, clays, quartz, feldspars and metal sulfides measured by XRD for Rudna carbonate ore.

The largest group of minerals is carbonate minerals which account for 60% of the total weight. The minor groups of gangue are clay minerals and quartz with the 13% and 8% of the weight respectively. Feldspars make up only 3% of the ore. The feed ore contains 4% of copper sulfides.

3.6.1 Leaching with PGA

Figure 36 illustrates leaching results with the highest copper yield among the experiments using PGA as a leaching agent for Rudna Carbonate ore.

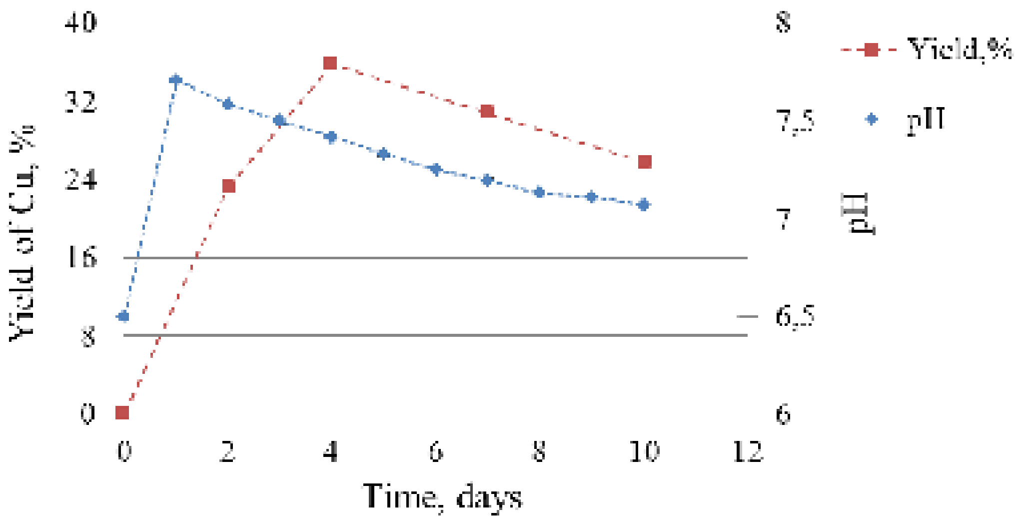


Figure 36 Leaching kinetics of Rudna Carbonate by PGA

The leaching was performed in Erlenmeyer flasks (refer 2.6.1) at 27°C. Samples were taken at 2nd, 4th, 7th and 10th day and pre-treated as previously described (refer 2.6.2) in order to determine the yield of copper in Rudna Carbonate ore (-90µm) in the cell-free supernatant of *Bacillus licheniformis* with PGA concentration of 12 g/l. The blue line shows pH change over 10 days and the red line copper yield.

The line Figure 36 illustrates the leaching kinetics of copper from Rudna Carbonate ore over a 10 day period and the pH alteration of the leaching solution. The highest Cu yield among experiments with PGA, was reached with the following conditions:

Parameter	Values
Pulp density	15 %
Temperature	31 °C
pH	6.5

After beginning the experiment, the yield steeply increased to 23.2% in two days, and showed further growth on Day four, reaching the peak with the yield of 33.5%. Then, the yield gradually went down to 30.8% and decreased to 25.8% on the Day ten.

In Figure 36, the second line (blue) demonstrates the pH change during the experiment. The initial pH of the culture broth was 6.5. On the Day one, there was a dramatic jump of the pH to 7.71. A steady decrease was experienced to a pH of 7.07 on Day 10.

Due to statistical significance value above the limits of 0.05, the response surface model may not be considered as a reliable and excluded from the results.

3.6.2 Leaching with stepwise change of supernatant

In order to examine the influence of pretreatment with biologically produced organic acids and ammonium before leaching with PGA experiment with a stepwise change of leaching supernatant was implemented.

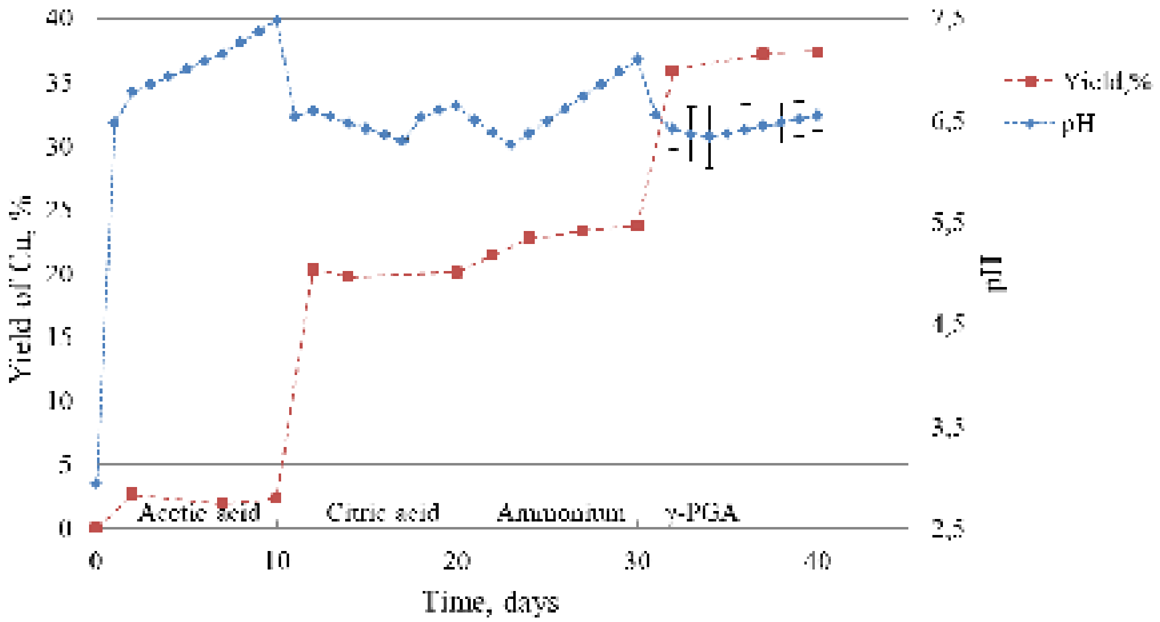


Figure 37 Leaching kinetics of Rudna Carbonate with stepwise change of supernatant

Rudna Carbonate (-90µm) leaching with a stepwise change of cell-free supernatant containing defined amount of biologically produced leaching agents such as acetic acid by Kombucha-culture, citric acid (6.5 g/l) by *Yarrowia lipolytica*, ammonium (0.5-0.6 g/l) by *Sporosarcina ureae* and PGA by *Bacillus licheniformis*. The blue line shows pH change over 40 days. Experiment run in an overhead mixer with pulp density = 15 % and lab temperature.

The line Figure 37 illustrates the leaching kinetics of Cu from Rudna carbonate ore over 40 days, and the pH alteration of the leaching solution. The experiment was run with the sample duplicate. Thus, the points are the mean value and the error bars at every measurement point show the standard deviation of a particular value.

In Figure 37, the time axis indicates that the main leaching compounds were utilized in the leaching experiment within a certain time span. During the first 10 days of leaching experiment, the Kombucha culture broth was applied. This resulted in the copper yield of 2.7% on the second day. A plateau was experienced until the Day 10 with the yield of 2.4%.

After PLS was removed, Mansfeld copper shale ore was washed out with distilled water and mixed with cell-free culture broth of *Yarrowia lipolytica,* which contains 6.5 g/l of citric acid. Following this addition, the experiment was run for ten more days (Days 10 - 20). The yield is cumulative and shows the sum of yields on the last day of leaching with acetic acid (Day 10) and the yield caused by leaching with citric acid on second, fourth, seventh and tenth days. The yield went up significantly and on Day 2, reaching a copper recovery rate of 20.3%. Then the yield remained stable with the yield of 20.1% on Day 10.

After ten days of leaching with citric acid, the same PLS removal and washing procedures were applied, and the culture broth of *Sporosarcina ureae* was added with the ammonium concentration of 0.5-0.6 g/l. The experiment was run for another 10 days (Days 20-30). The yield is cumulative and shows the sum of yields at the last day of leaching with culture broth of *Yarrowia lipolytica* (day 20) and the yield caused by leaching with ammonium on second, fourth, seventh and tenth days.
The yield remained stable during the experiment. On the Day 30, an overall yield of 23.8% after 10 days of leaching with ammonium was reached.

The culture broth of *Bacillus licheniformis* has a PGA content of 12-13 g/l and was used on Days 30-40 of the leaching experiment.
The copper yield dramatically went up on the second day of leaching with PGA reaching 35.9% and then increased to 37.4% on Day 40.

During the experiment, the pH rapidly increased from 2.94 to 7.4 on Day 10. This is due to the high neutralization potential of carbonates in the ore and tended to be within the limit of 6.2-7 varying slightly over next 30 days.

3.6.3 Mineralogical analyze of copper mineral

In order to examine an impact of applied biologically produced leaching agents on mineral content, copper sulfides were measured before and after leaching experiment.

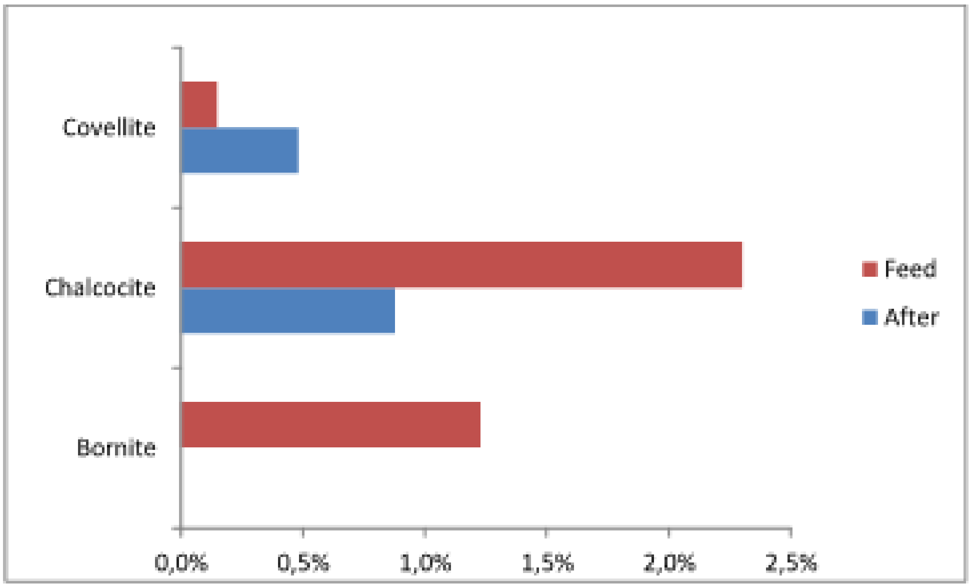


Figure 38 Mineralogical analyses of copper sulfides in Rudna carbonate before and after leaching

Mineralogical analyses of copper sulfides were performed by MLA at 25 keV for feed Rudna carbonate ore and for remaining solid ore sample after leaching with a stepwise change of supernatants (refer 2.6.2). Minerals content are overestimated (refer discussion) but provides the qualitative characteristic of leaching efficiency with respect to copper minerals. Not identified measured area is 11.5% (mostly due to mixed spectra from multiple minerals)

Figure 38 demonstrates the changes in detected copper minerals in feed ore and remaining copper sulfides after leaching. Quantitative distribution of the minerals are not realistic due to overestimation but provides an overview if the mineral became dissolved, remain undissolved or mineral formation took place during the leaching experiment. Due to overestimation, only the trends are interpreted without numerical consideration.

According to MLA results, chalcocite which represents more than a half of the copper sulfides was dissolved by about 70%, bornite content is noticeable and was entirely dissolved. After leaching the weight of chalcocite decreased by about 60% and some amount of covellite additionally formed during the leaching.

4. Cumulative discussion

Copper shale ore possesses a high content of valuable metals according to Kucha, 1990 such as Cu, Zn, Pb, Ag, Au, Ga etc. that has been proved with ICP-MS measurements, and the yield of this elements was measured within all tests but as long as Cu yield showed much higher extraction rate comparing the accompanying elements research results dedicated to copper leaching. Especially in Mansfeld ore the present value of silver at least 2 times more than copper, in Sangerhausen copper shale Ga is the most valuable recovery but with examined leaching agents showed disappointingly low yield (typically less than 3%) and not indicated in the results.

The results demonstrate an effect of biologically produced leaching agents such as acetic acid, citric acid, ammonium and PGA on the solubilization of copper from six copper shale ore samples. As long as organic acids capable of forming complexes with metals CA acetic acid (Kostudis, 2015), ammonium (Chmielewski, et al., 2009) and based on previous studies the attempts were put on process optimization in order to increase metal recovery from the ore.

Copper shale ore possesses high carbonate content which involves high neutralization potential that inhibits traditional bioleaching. There is an opportunity to remove the carbonates by acid pre-treatment in order to avoid gypsum formation which inhibits leaching and then copper minerals can be leached out from the ore (d'Hugues, et al., 2008). Results of traditional bioleaching of copper shale ore with acidophilic microorganisms reported to be efficient with the yield more than 70 % (Sethurajan, et al., 2012)but require lowering the pH to <2 by external addition of acid. For instance, the Rudna carbonate ore sample contains 60% of carbonates by weight, therefore before leaching of copper minerals under acidic conditions more than a half of the feed ore that represents carbonates should be removed. That leads to an investigation of alternative leaching approaches.

Another opportunity is leaching under neutral or alkaline pH which allows carbonate minerals remains intact in the ore dissolving only the copper minerals. Leaching under this conditions involves issues such as minerals solubility, leaching kinetics, chemical complexation process, new mineral formation etc. that have to be further investigated.

In the study (Schwartz & Kiel, 1980) the yield of copper from copper shale ore reached as much as 87.3% within 2 weeks using heterotrophic microorganism *Aspergillus niger* that capable of excreting citric acid. Comparison of the copper solubilisation from different literature sources limited by various ore types and different mineral composition. Even in a present study where the ore samples come from European copper basin the results varies dramatically depending on mineral composition. For instance, leaching experiments showed the recovery of 45.1% of copper in Mansfeld copper shale and 17% in Rudna shale using the same condition of the experiment that indicates a strong influence of mineralogy that also was investigated.

Due to heterotrophic bioleaching involves indirect mechanism (Rezza et al. 2001; Bosecker, 1997) the microorganisms cells were removed before leaching to prevent further growth and change in lixiviants concentration.
Two different experiments configuration were chosen in order to see the sole influence of PGA (refer 2.6.1) on copper yield and influence of pre-treatment leaching steps (refer 2.6.2). In the literature was reported that CuO dissolution by an organic acid such as citric acid highly temperature dependent reaching a copper yield of 32% at 40 °C and 99% at 80 °C, even though copper oxide leaching was examined the temperature might be considered as a crucial factor in copper sulfides leaching. (Habbache, et al., 2009)

An interesting fact that leaching by PGA over 10 days showed almost the same results as an experiment with ore pre-treatment by acetic acid, citric acid and ammonium for 30 days before leaching by PGA for 10 days that indicates all of the applied lixiviants possess similar leaching properties.
The copper yield can be explained by a change in ore minerals composition, therefore minerals were measured before and after leaching by MLA and XRD. The copper sulfides bioleaching kinetics was reported to vary depending on mineral species chalcocite>bornite>covellite>chalcopyrite (Kutschke, et al., 2015).

Eventually leaching with organic acids, ammonium and PGA showed a dramatic increase of copper yield in first 2 days that can be linked to chalcocite dissolution, then steep increase was observed that may include bornite dissolution. A small part of covellite was formed that often reported in the literature (Ahonen & Tuovinen, 1995).

A small part of chalcopyrite was leached out of Rudna sandstone and Rudna copper shale even though it characterized as a refractory mineral (Córdoba, et al., 2008) that might reveal the higher ability of chalcopyrite to be dissolved at neutral pH than at low. That can be supported by stability diagram (Figure 39) which shows chalcopyrite stability at higher redox potential in the pH range of 1-3 in comparison to pH of 6-8.

Considering the mass balance of the extracted metal important to mention that after 40 days of leaching big parts of chalcocite and bornite were dissolved that can be proved by the statement that chalcocite and bornite are easiest to leach copper sulfide (Chmielewski, et al., 2009). But such an enormous mineral dissolution is controversial with accordance to theoretically released copper metal. As long as chalcocite and bornite are the main copper minerals in copper shale ore the entire dissolution of those minerals means that Cu yield for Rudna sandstone is 64%, 54% in Rudna shale and 62% in Rudna carbonate. Meanwhile, extracted Cu into solution measured by ISP-MS demonstrates 29.4% of yield in Rudna carbonate, 17% of yield in Rudna shale and 37.4% of yield in Rudna carbonate.

Such a large difference between cooper yield can be explained either by classification of copper sulfides after leaching as an unknown fraction during MLA data processing or by the formation of new minerals other than sulfides.

In the work (Córdoba, et al., 2008) illustrated Pourbaix diagram for the $CuFeS_2$-H_2O system which reveals transformation possibility of copper sulfides to Cu^0, Cu_2O at neutral pH end Eh greater than about 0.7 that seems to be operated conditions of performed experiments (Figure 39). Worth to mention that once after not acidified leftover PLS samples storage at lab temperature over the weekend the walls of the sampling tubes were covered with metal like a thin layer that could be elemental copper formation (Figure 40). The same Cu^0 or even Cu_2O formation could take place during the leaching but was not noticed in the slurry. MLA was targeted to copper sulfides identification that could not identify copper oxides or elemental copper after leaching.

5. Conclusion

In the present thesis optimization of the leaching process was investigated that allowed to reveal optimal leaching conditions and presumed to enhance copper yield up to 3.3% by experiments planning technique. Chemical analyses showed that only limited amount of copper can be dissolved depending on ore sample under applied conditions and mineralogical analyses demonstrated which minerals were dissolved or formed. Despite the usage of optimization technique, the copper yield did not reach more than 43.9% but the wide range of altered parameters gives an overview on the effect of their variation.

Two different experiment's types such as leaching with cell-free culture broth of *Bacillus licheniformis* over ten days and experiment with a stepwise change of leaching supernatants produced by Kombucha-culture, *Yarrowia lipolytica* and *Sporosarcina ureae* over forty days extracted into solution almost the same amount of copper. Referring to the measured yields and mineralogical analyses let conclusion that none of the copper sulfides are able to be dissolved entirely under applied conditions. But a significant amount of chalcocite and bornite was not more in the form of minerals prove the leaching effect even though the dissolution stops at some point. Some copper shale ore samples contained a small portion of chalcopyrite which is refractory during bioleaching with acidophilic microorganisms but after leaching at neutral pH chalcopyrite was partly dissolved. All experiments demonstrate an increase in covellite content that confirms the formation of this mineral.

Utilization of biologically produced PGA in 10 days extracted more copper from 4 out of 6 ore samples comparing to experiment with pre-treatment leaching steps with acetic acid, citric acid and ammonium containing supernatants. That proves that among tested leaching agents PGA possesses the highest leaching ability that can be further investigated.

Even though relatively low copper yield that was reached, does not allow implementing the process in industrial scale yet, the change of leaching conditions may increase process efficiency. There are well-known techniques which are able to boost mineral dissolution and at the same time accelerate process kinetic such as pressure leaching, temperature elevation, leaching under oxidative conditions etc.

Bibliography

Ahonen, L. & Tuovinen, O. H., 1995. Bacterial leaching of complex sulfide ore samples in bench-scale column reactors. *Hydrometallurgy* , Volume 37(1), pp. 1-21.

Alderton; David Selby; Henrik Kucha; Derek J. Blundell, 2016. A multistage origin for Kupferschiefer mineralization. *Ore Geology Reviews 79,* p. 535–543.

Ashiuch, 2013. Microbial production and chemical transformation of. *Microbial Biotechnology,* p. 664–674.

Ashiuchi & Makoto, 2011. Ashiuchi, Analytical approaches to poly-γ-glutamate. Quantification, molecular size determination, and stereochemistry investigation. *Journal of Chromatography,* Volume 879 (29), p. 3096–3101.

Bajaj, S., R. & Singhal, 2009. Sequential Optimization Approach for Enhanced Production of Poly(g-Glutamic Acid) from Newly Isolated Bacillus subtilis. *Food Technol. Biotechnol. 47 (3),* p. 313–322.

Bautista, 1984. *Hydrometallurgical Process Fundamentals.* New Yourk: Springer science + Business media.

BGS, 2007. *British Geological Survey, Copper,* s.l.: NERK.
Borg, et al., 2012. *An Overview of the European Kupferschiefer Deposits.* BORG ed. s.l.:Society of Economic Geologists.

Bosecker, K., 1997. Bioleaching: metal solubilization by microorganisms. *FEMS Microbiology 20,* pp. 591-604.

Cavazzuti, M., 2013. *Optimization Methods: From Theory to Design.* Modena: Springer-Verlag Berlin Heidelberg.

Chang, (仲兆祥), Z. Z., (徐虹), X. H. & (姚忠), Y. Z., 2013. Fabrication of Poly(γ-glutamic acid)-coated Fe3O4 Magnetic Nanoparticles and Their Application in Heavy Metal Removal. *Chinese Journal of Chemical Engineering 21,* p. 1244—1250.

Chiang, Y. W. et al., 2013. Effects of bioleaching on the chemical, mineralogical and morphological properties of natural and waste-derived alkaline materials. *Minerals Engineering 48,* p. 116–125.

Chmielewski, Wódka, J. & Iwachów, Ł., 2009. AMMONIA PRESSURE LEACHING FOR LUBIN. *Physicochemical Problems of Mineral Processing,* pp. 5-20.

Clarke, F. &. W. H., 1924. *The Composition of the Earth's Crust.* WASHINGTON: UNITED STATES GEOLOGICAL SURVEY.

Córdoba, E. et al., 2008. Bacterial leaching of complex sulfide ore samples in bench-scale column reactors. *Hydrometallurgy,* 93(3–4), p. 81–87.
d'Hugues, P. et al., 2008. Bioshale FP6 European project: Exploiting black shale ores using biotechnologies?. *Minerals Engineering,* Volume 21 , p. 111–120.

Dunbar, W. S., 2015. *How mining works.* Englewood, Colorado: Society for Mining, Metallurgy & Exploration (SME).

Edelstein, D. L., 2012. *Mineral Yearbook 2010,* Washington: U.S. Geological Survey.
EUreport, 2014. *NON-CRITICAL RAW MATERIALS PROFILES,* s.l.: DG Enterprise and Industry.

Free, M. L., 2013. *Hydrometallurgy : fundamentals and applications.* Hoboken, New Jersey: John Wiley & Sons, Inc..

Free, M. L., 2014. Biohydrometallurgy. In: *Treatise of Process Metallurgy.* Oxford, UK: Elsevier, pp. 983-994.

FU, C. et al., 2014. Antioxidant activities of kombucha prepared from three different substrates and changes in content of probiotics during storage. *Food Science and Technology,* Volume 34(1), pp. 123-126.

Goh, et al., 2012. Fermentation of black tea broth (Kombucha): I. Effects of sucrose concentration and fermentation time on the yield of microbial cellulose. *International Food Research Journal ,* Volume 19(1), pp. 109-117.

Goldman, Gruninger & Manuel, 1988. Evidence for urea cycle activity in Sporosarcina ureae. *Archives of Microbiology 150,* pp. 394- 399.

GREWAL, H. & KALRA, K., 1995. FUNGAL PRODUCTION OF CITRIC ACID. *Biotechnology Advances,* Volume 13, pp. 209--234.

Groudeva, Krumova, K. & Groudev, S. N., 2007. V.I. Groudeva, K. Krumova, S. N. Groudev, "Bioleaching of a Rich-In-Carbonates Copper Ore at Alkaline pH. *Advanced Materials Research,* pp. 103-106.

Habashi, F., 1997. *Handbook of Extractive Metallurgy (Vol. 2).* Toronto: Wiley-VCH.
Habbache, N., Alane, N., Djerad, S. & Tifouti, L., 2009. Leaching of copper oxide with different acid solutions. *Chemical Engineering Journal,* Volume 152 , p. 503–508.

Han & Meng, 1996. The Principles and Applications of Ammonia. *Mineral Processing and Extractive Metallurgy,* pp. 23-61.

HISKEY, ONER, G. & COLLINS, D. W., 1992. Recent Trends in Copper In Situ Leaching. *Mineral Processing and Extractive Metallurgy,* pp. 1-16.

Institute, P. G., 2016. *PGI,* s.l.: Polish Geologicsl Institute.
Jayabalan, et al., 2010. Biochemical Characteristics of Tea Fungus Produced During. *Food Sci. Biotechnol. ,* Volume 19(3), pp. 843-847.

Kaczmarczyk & Lochyński, S., 2014. PRODUCTS OF BIOTRANSFORMATION OF TEA INFUSION – PROPERTIES AND APPLICATION. *POLISH JOURNAL OF NATURAL SCIENCES,* Volume Vol 29(4), p. 381–392.
Knutsen, et al., 2007. Polyphasic re-examination of Yarrowia lipolytica strains and the description of three novel Candida species: Candida oslonensis sp. nov., Candida alimentaria

sp. nov. and Candida hollandica sp. nov.. *International Journal of Systematic and Evolutionary Microbiology,* Volume 57, p. 2426–2435.

Kostudis, K. B. S. K. K. P. J. G., 2015. Leaching of copper from Kupferschiefer by glutamic acid and. *Minerals Engineering 75,* p. 38–44.

KUCHA, H., 1990. Geochemistry of the Kupferschiefer, Poland. *Geologische Rundschau 79/2,* pp. 387-399.

Kutschke, et al., 2015. Bioleaching of Kupferschiefer blackshale – A review including. *Minerals Engineering 75,* p. 116–125.

Liu & Chen, 2000. Changes in major components of tea fungus metabolites during prolonged fermentation. *Journal of Applied Microbiology,* Volume 89, pp. 834-839.

Lossin, A., 2001. *Ullmann's Encyclopedia of Industrial Chemistry.* s.l.:Wiley-VCH Verlag GmbH & Co. KGaA.

Margaritis, M. B. &. A., 2007. Microbial Biosynthesis of Polyglutamic Acid Biopolymer and Applications in the Biopharmaceutical, Biomedical and Food Industries. *Critical Reviews in Biotechnology, 27,* p. 1–19.

Marsh, A. J. et al., 2014. Sequence-based analysis of the bacterial and fungal compositions of multiple kombucha (tea fungus) samples. *Food Microbiology,* Volume 38 , pp. 171-178.

McLean, R. J. C., Beauchemin, D., Clapham, L. & Beveridge, T. J., 1990. Metal-binding characteristics of the gamma-glutamyl capsular polymer of Bacillus licheniformis ATCC 9945. *Applied and environmental microbiology 56 (12),* p. 3671–3677.

Megazyme, 2016. [Online]
Available at: https://secure.megazyme.com/files/Booklet/K-AMIAR_DATA.pdf
[Accessed 14 09 2016].

Mitsunagai, M. L. P. T. G. B. T., 2016. Metabolome analysis reveals the effect of carbon catabolite control on the poly(γ-glutamic acid) biosynthesis of Bacillus licheniformis ATCC 9945. *Journal of bioscience and bioengineering.*

MULLER, B., KLAGER, W. & KUBITZKI, G., 1997. METAL CHELATES OF CITRIC ACID AS CORROSION INHIBITORS FOR ZINC PIGMENT. *Corrosion Science,* Volume 39, pp. 1481-1485.
Natarajan, C. &. K. A., 2010. Microbially Induced Mineral Beneficiation. *Mineral Processing & Extractive Metall. ,* p. 1–29.

Nicaud, J.-M., 2012. Yarrowia lipolytica. *Yeast,* Volume 29, p. 409–418.
Oustan, S. et al., 2011. *Removal of heavy metals from a contaminated calcareous soil using oxalic and.* Singapore, IACSIT Press.

Preißler, S., 2016. *Optimierung der bakteriellen Polyglutaminsäureproduktion zur Laugung von Kupferschiefer,* Dresden: Masterarbeit.
Priest, F. G., 1993. *Systematics and Ecology of Bacillus.* Washington, DC.: ASM Press.

Radmehr, V. K. S. K. M., 2013. Ammonia Leaching: A New Approach of Copper Industry in Hydrometallurgical Processes. *Journal of The Institution of Engineers (India),* p. 95–104.

Ramachandran, S., Fontanille, P., Pandey, A. & Larroche, C., 2006. Gluconic Acid: Properties, Applications and Microbial Production. *Food Technol. Biotechnol.* , Volume 44 (2), p. 185–195.

Reichl, M. S. G. Z., 2016. *WORLD-MINING-DATA,* Vienna: Federal Ministry of Science, Research and Economy.

Rezza, I., SALINAS, E. & ELORZA, M., 2001. Mechanisms involved in bioleaching of an aluminosilicate by heterotrophic microorganisms.. *Process Biochemistry 36,* pp. 495-500.

RIET, J. V. '., WIENTJES, F., DOORN, J. V. & PLANTA, R., 1979. PURIFICATION AND CHARACTERIZATION OF THE RESPIRATORY. *Biochimica et Biophysica Acta, 576,* pp. 347--360.

Scheper, Schippers, A., Glombitza, F. & Sand, W., 2014. *Geobiotechnology I.* Hannover: Springer-Verlag Berlin.

Schippers & Sand, 1999. Bacterial Leaching of Metal Sulfides Proceeds by Two Indirect. *APPLIED AND ENVIRONMENTAL MICROBIOLOGY,* p. 319–321.

Schwartz & Kiel, 1980. Leaching of a silicate and carbonate copper ore with heterotrophic fungi and bacteria, producing organic acids. *Arbeitsgruppe Geomikrobiologie am Lehrstuhl fiir Mikrobiologie.*

Sethurajan, M., Aruliah, R. & Karthikeyan, O. P., 2012. BIOLEACHING OF COPPER FROM BLACK SHALE USING MESOPHILIC MIXED POPULATIONS IN AN AIR UP-LIFT BIOREACTOR. *Environmental Engineering and Management Journal,* Volume 11, pp. 1839-1848.

Shariati, Mitchell, W. J., Boyd, A. & Priest, F. G., 1995. Anaerobic metabolism in Bacillus licheniformis. *Microbiology,* pp. 1117-1124.

Suna, M.-L.et al., 2016. Engineering Yarrowia lipolytica for efficient linolenic acid production. *Biochemical Engineering Journal* , Volume 117 , p. 172–180.

USGS, 2013. *Commodity Summaries: Copper,* s.l.: US Geological Survey.

Appendix

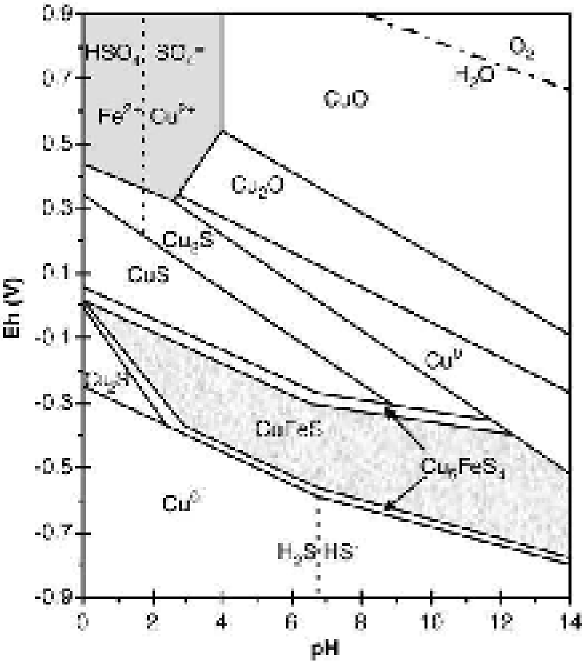


Figure 39 Pourbaix diagram for the CuFeS2-H2O system (Córdoba, et al., 2008)

Figure 40 PLS samples