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Effects of Ultraviolet Irradiation on Bacterial (Acidithiobacillus Ferrooxidans) Mutation and Bioleaching of Refractory Gold Ores from the Ovacik Gold Mine, Turkey

Seda Demirci[1], Oktay Bayat[2]
[1,2] *Cukurova University, Vocational School of Aladag, , Adana, Turkey*

Abstract: ***Effects of ultraviolet (UV) irradiation on mutation rates in* Acidithiobacillus ferrooxidans *bacteria and subsequent bio-oxidation of refractory gold ores from the Ovacik Mine (Koza Gold Co.) in Turkey were examined. UV radiation-induced mutations improved bio-activity, and gold bio-oxidation by mesophilic bacteria was greater at lower pulp densities and in the presence of iron than in an iron-free solution. Moreover, UV-mediated mutations improved bio-activity and bioleaching capabilities. Specifically, after 5-min UV irradiation, the dissolution rate for iron in 1% solids was 99.17% in the presence of* A. ferrooxidans *in FeTSB medium.***

***Keywords*— A. ferrooxidans, Ultraviolet, Mesophilic bacteria, FeTSB medium, Gold mine**

I. INTRODUCTION

Refractory gold ore is resistant to gold recovery by direct cyanidation and carbon adsorption. However, because of increasingly limited availability of gold rich ores, gold mining companies are switching towards low-grade, refractory gold-bearing ores [1]. Biotechnology has the potential to transform uneconomic Au reserves into mineable resources. It can be attractive for; i. low grade Au ores that are uneconomical to process using conventional metallurgical techniques, and ii. refractory Au ores requiring oxidation of the sulfides to liberate the gold. The bio-oxidation is a pre-treatment process which can decrease the consumption of chemical lixiviants, such as cyanide, for Au solubilisation in subsequent parts of the operation and ultimately increase Au yields [2]. Cyanidation to recover gold from ore or secondary resources has been in use almost universally based on formation of gold-cyanide complex which shows very good water solubility and high chemical stability [3]. However, excessive use of cyanide for the dissolution of gold is associated with environmental risk, and thus biological methods for gold leaching are being investigated as environmental friendly methods [4]. The acidophilic chemolithotrophic gram-negative bacterium Acidithiobacillus ferrooxidans is a predominant microorganism in commercial bio-oxidation industries, and it harnesses energy from the oxidation of ferrous to ferric ions using molecular oxygen as the terminal electron acceptor under aerobic conditions [5]. Previous studies show that acidophilic iron-oxidizing bacteria such as Thiobacillus ferrooxidans and Leptospirillum ferrooxidans are widely distributed [6] and are considered to be the most important mesophiles for the extraction of metals from sulfidic ores [7]. Although T. ferrooxidans have been intensively studied for application to hydrometallurgical extraction of metals from sulphidic ores [8-11], Norris et al. [1987] reported that L. ferrooxidans dissolved pyrite more extensively than T. ferrooxidans. Subsequently, T. ferrooxidans was shown to oxidize framboidal pyrite, but not euhedral pyrite, and L. ferrooxidans was shown to oxidize both pyrite resources [13]. However, T. ferrooxidans oxidizes both ferrous iron and reduced sulfuric compounds, whereas L. ferrooxidans only oxidizes ferrous iron [14]. Prominent bacterial breeding methods include domestication mutagenesis and genetic engineering [15]. Although breeding of leaching bacteria has been investigated worldwide, some challenges remain in producing excellent strains for industrial applications, and high efficiency strains are extremely scarce because of inadequate oxidation capacity and susceptibility to adverse environments. Moreover, techniques for domestication of strains are time-consuming and inefficient and genetic engineering approaches remain poorly developed. Therefore, mutation breeding is the most popular method for enhancing bioleaching activities of ferrooxidans strains. UV induced mutation is the simplest and most effective physical mutation method, and is most effective at wavelengths of approximately 255 nm that correspond with the absorption spectrum of bacterial DNA [16]. The bio-oxidation is a pre-treatment process which can decrease the consumption of chemical lixiviants, such as cyanide, for Au solubilisation in subsequent parts of the operation and ultimately increase Au yields. However, since it does not actually solubilise Au, bio-oxidation

needs to be used in conjunction with other methods [17]. The use of non-conventional lixiviants, such as thiosulfate, has recently received attention as an alternative technology to the cyanidation of gold ores due to the growing environmental and public concerns over the use of cyanide [18-19]. Oxide Au ores are well suited for thiosulfate leaching, while sulfide ores show low extractions. In refractory Au ores fine patches of gold are often locked in the sulfide matrix. In order to recover gold, high sulphide containing ores require at least partial oxidation prior to thiosulfate leaching [18].

In this present study, the effects of UV-induced mutation in A. ferrooxidans on oxidation activities were investigated and bioleaching of refractory low-grade gold ores from Ovacik, Turkey was evaluated with various experimental conditions such as pH, pulp density and bio-oxidative treatment along with the mineralogical studies [20].

II. MATERIAL AND METHODS

A. Microbial strain and culture conditions

The A. ferrooxidans strain used in this study was obtained from DSMZ (Deutsche Sammlung von mikroorganismen und Zellkulturen) and was grown in an optimized 9K medium comprising 0.4-g/dm^3 $(NH_4)_2SO_4$, 0.2-g/dm^3 K_2HPO_4, 0.4-g/dm^3 $MgSO_4.7H_2O$, 0.1-g/dm^3 KCL and 55.6-g/dm^3 $FeSO_4.7H_2O$. The pH of the culture media was adjusted using sulphuric acid (98%) and cultures were maintained in basal medium at pH 1.7. Maximum growth of the cells was achieved after 72 h at 30°C in an orbital agitator at 150 rpm. Where necessary, 5% (v/v) inoculums of active cultures were used and cell suspensions were initially passed through a Whatman filter to remove precipitates. Filtrates were centrifuged at 4,200 rpm for 30 min, and the resulting pellets were re-suspended in a solution of sulphuric acid at pH 2.0 and placed in a refrigerator until precipitates had settled. Supernatants were re-centrifuged as described above and were then repeatedly washed with sulphuric acid until the cells were free from iron [21]. The cells were suspended in 10 cm^3 of acid solution at pH 2.0 and were stored at 30°C. These bacteria remained active for at least 1 month under these conditions.

B. Gold Samples

Gold samples were obtained from the Ovacik Gold Mine (Koza Gold Co.) in Turkey and chemical analyses were performed by X-ray fluorescence (XRF) spectrometer (Table 1). X-ray diffraction (XRD) analyses were conducted to identify mineralogical characteristics of the ore and showed predominance of hematite (Fe_2O_3) and quartz (SiO_2; Fig. 1). Accordingly, mineralisation at the Ovacik Gold Mine is a vein type, and the ore is collected from a low sulphidation epithermal deposit comprising two economically viable epithermal quartz veins formed in andesitic volcanic rocks.

TABLE I.
CHEMICAL ANALYSIS OF THE ORES USED IN THIS STUDY (MASS FRACTION, %)

CO_2	Na_2O	MgO	Al_2O_3	SiO_2	P_2O_5	Fe_2O_3	NiO	Rb_2O
4.84	0.122	0.353	3.60	86.1	0.0587	1.84	0.0336	0.0088
SO_3	K_2O	CaO	TiO_2	Cr_2O_3	MnO	Sb_2O_3	SrO	Au
0.0690	2.14	0.422	0.0876	0.220	0.0286	0.0545	0.0087	0.0135

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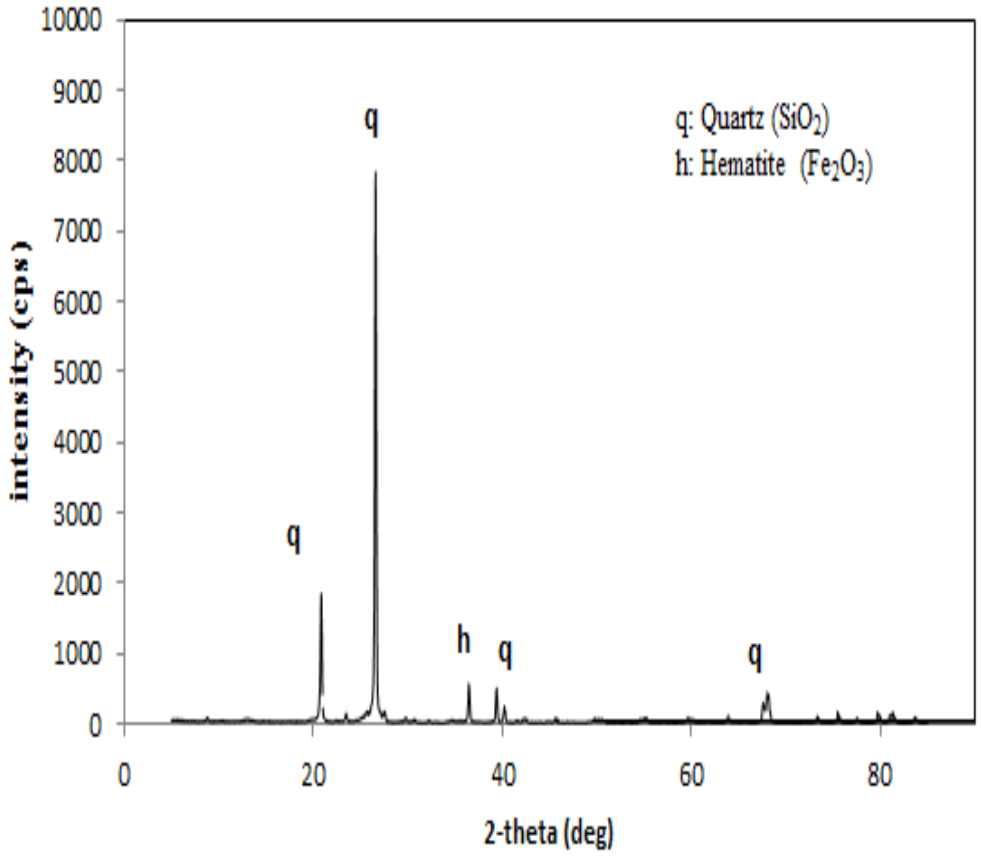

Fig. 1. XRD pattern of the ore

C. Mutation

Mutation experiments were performed using a 15-W UV lamp with a wavelength of 254 nm. Bacteria were cultured in FeTSB (40g/L $FeSO_4.7H_2O$, 1,8g/L $(NH_4)_2SO_4$, 0,7g/L $MgSO_4.7H_2O$, 0,35g/L Triptik soy agar, 14g/L Agarose), ISP (33,4g/L $FeSO_4.7H_2O$, 6 g/L $(NH_4)_2SO_4$, 1g/L $MgSO_4.7H_2O$, 0,2/L g KCI, 0,02g/L $Ca(NO_3)_2.4H_2O$, 7g/L Agar) agar media during mutagenic UV exposures, and only FeTSB and ISP mediums showed mutant bacteria reproduction. However, in subsequent experiments viable bacterial colonies were not present in ISP media after 5-min UV exposures, but could be counted on FeTSB agar under the same conditions. These data indicate that bacteria oxidize Fe^{2+} more efficiently in FeTSB than in the absence of free-iron. Suspended cells, *A. Ferrooxidans*, were cultured on plates and irradiated at a distance of 30 cm for 1, 2, 3, 5, 10, 15 and 20 min. Numbers of mutant bacteria were counted under a microscope, and bacterial mortality rates were calculated for each exposure time. No mutant bacteria were detected after 10-min exposures to UV. After mutation, samples were isolated from light and stored in a refrigerator at 4°C for 12 h to prevent recovery. Subsequently, bacterial mutants were cultivated in 9K liquid medium under optimal growth conditions and cell densities and Fe^{2+} oxidation rates were measured every 12 h [16]. Bacterial mutants with good Fe^{2+} oxidation activity were selected for gold ore bio-oxidation experiments, and those growing in the stationary growth phase in 9K medium after 7 days were collected by centrifugation at 10,000 rpm/min for 20 min and were washed with distilled water and finally suspended in acetate buffer.

D. Bio-oxidation experiments

Bio-oxidation tests were performed at 1%, 3%, 5% and 10% pulp densities (w/v) using gold ore samples in 500-cm^3 Erlenmeyer flasks containing 350 cm^3 of 9K medium (pH 1.8 ± 0.05). Flasks were inoculated with 3.5 cm^3 (27×10^6 cell/cm^3) of bacterial cell suspension and were incubated for 19 days. Shake flasks were maintained at 30±2 °C at 150 rpm. Numbers of viable bacteria, pH, redox potentials, temperatures and iron concentrations in bio-oxidation solutions were determined at regular intervals and atomic absorption spectrometry (AAS) analyses were performed to determine percentages of iron leached into the liquid medium. Control

experiments were performed in the absence of microorganisms. Aseptic conditions were maintained throughout the experiment Note that a single space must be inserted before and after a mathematical operators.

E. Analytical Methods

Concentrations of dissolved ferrous ions in leaching solutions were analyzed using AAS and pH values and redox potentials were measured using a WTW S-720 pH/Eh process controller. Oxidation properties of bacteria were then expressed as oxidation rates of ferrous ions. After bio-oxidation experiments, iron (Fe^{2+}) dissolution rates were calculated using the following equation:

$$\left(\frac{Fe\ in\ the\ solution\left(\frac{mg}{dm^3}\right) \times dilution\ factor}{Fe\ in\ the\ sample\ (mg) + Fe\ in\ the\ bacteria\ solution\left(\frac{mg}{dm^3}\right) + Fe\ in\ the\ 9K\ medium}\right) \times 100\% \quad (1)$$

All reagents were of analytical grade, and de-ionized water was used in all experiments.

III. RESULTS AND DISCUSSION

A. Bacterial mortality rates

Previously, Dong et al. (2011) showed the utility of UV-induced mutations to harvest copper from ores. Accordingly, UV irradiation increases energy of inner elections and activates molecules. Although the energy of UV is approximately 3–5 ER and has insufficient penetrability to cause ionization, UV radiation can change the structure of DNA and causes DNA strand breakage, intermolecular cross linking within DNA and between nucleic acids and proteins, hydration of cytosine and uracil and formation of pyrimidine dimers. Among these, thymine dimers are the most common UV-induced DNA lesion, and can change the biological activity of DNA and cause bacterial mutations even after death. Moreover, high exposure to mutagens causes high mortality rates (approximately 90%–99%) with fewer positive mutant strains and more negative mutant strains among surviving cells. However, high efficiency strains can be selected from few positive mutant strains. In contrast, low exposures to mutagens are associated with only 50%–80% mortality, and more numerous positive mutant strains among surviving cells include a few high efficiency strains. UV mutagenesis follows changes in DNA that are caused by strong absorption of UV by base pairs. In particular, sensitivity of pyrimidine to UV is almost 100 times greater than that of purine. The double bond of thymine is converted into a single bond under UV light. Subsequently, circular bonds between thymine residues result in the formation of thymine dimers. Longer irradiation times result in increased numbers of newly formed thymine dimers, causing diversity of genetic variations and a high bacterial mortality.

In the present study, bacterial mortality rates during UV exposure were 17% after 10 min and 85% after 15 min. Hence, mortality rates were correlated with UV irradiation times and likely reflected the formation of thymine dimers. Thus, because irradiation times of >15 min caused approximately 100% mortality, subsequent bio-oxidation experiments were performed using mutant bacteria following 5-min UV exposures.

B. Shake flask bio-leaching study

The pH of culture solutions was determined before and after UV exposures, as shown in Figs. 2, 3, 4 and 5 respectively. Cultured bacteria are highly sensitive to pH, and optimal pH for growth and oxidation of Fe^{2+} is within a narrow range. The present experiments indicate that pH increases because of H^+ consumption by bacterial metabolism. Accordingly, during the first cultivation stage, Fe^{2+} oxidation was likely the main chemical reaction in the medium and was accelerated by bacteria, resulting in pH increases (1). However, at subsequent time points, Fe^{3+} concentrations in the medium likely increased and facilitated the following hydrolysis reaction (2):

$$Fe^{3+} + 3H_2O \longrightarrow Fe(OH)_3 + 3H^+ \quad (2)$$

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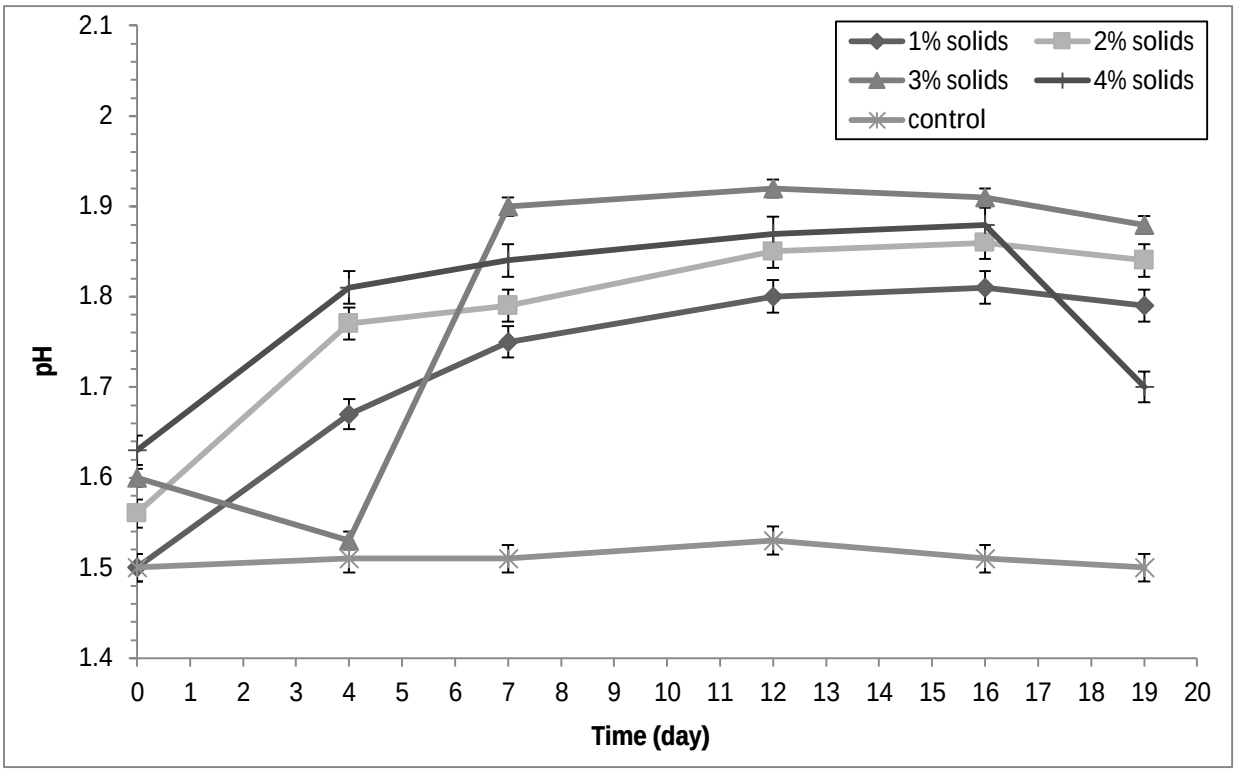

Fig 2. Changes in pH versus solids content in the presence of wild type bacteria

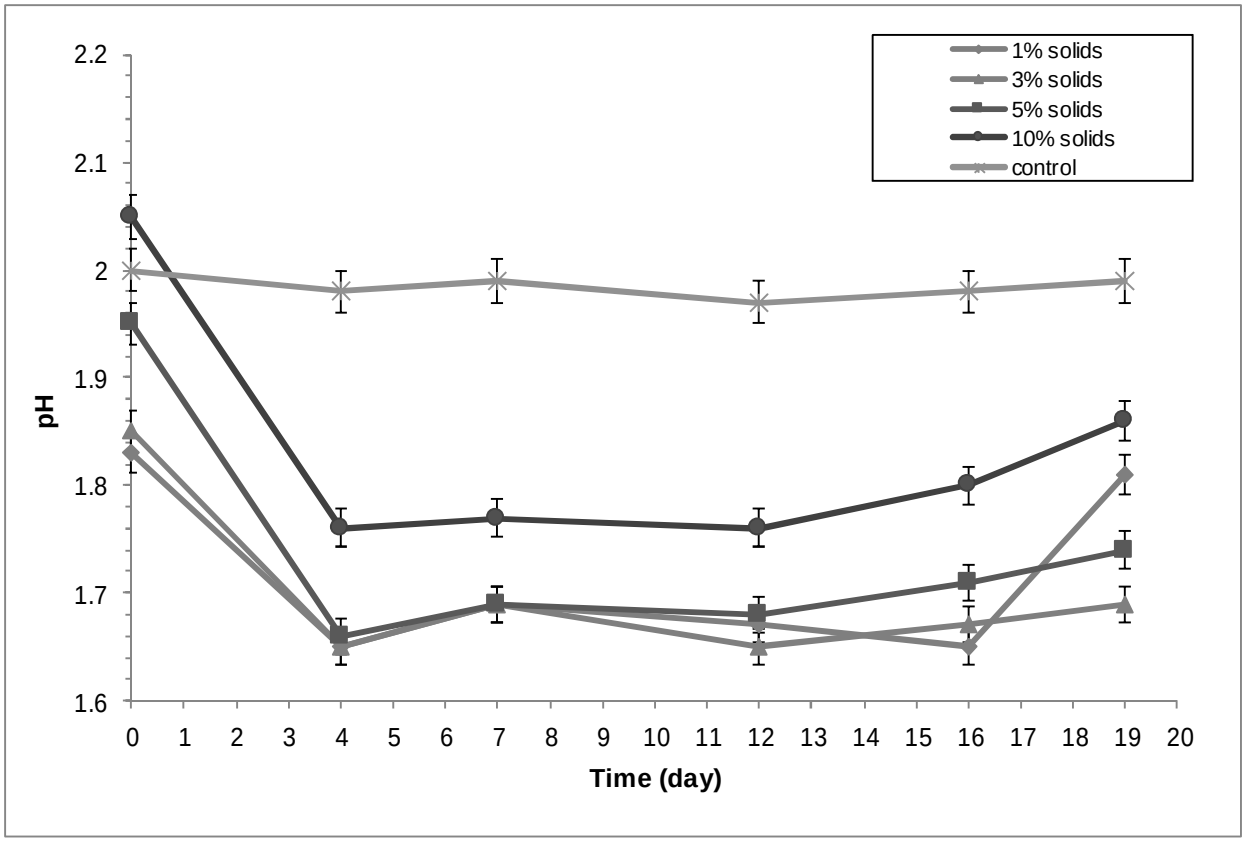

Fig 3. Changes in the pH versus solids content in the presence of wild type bacteria in 9K medium with the iron-free solution

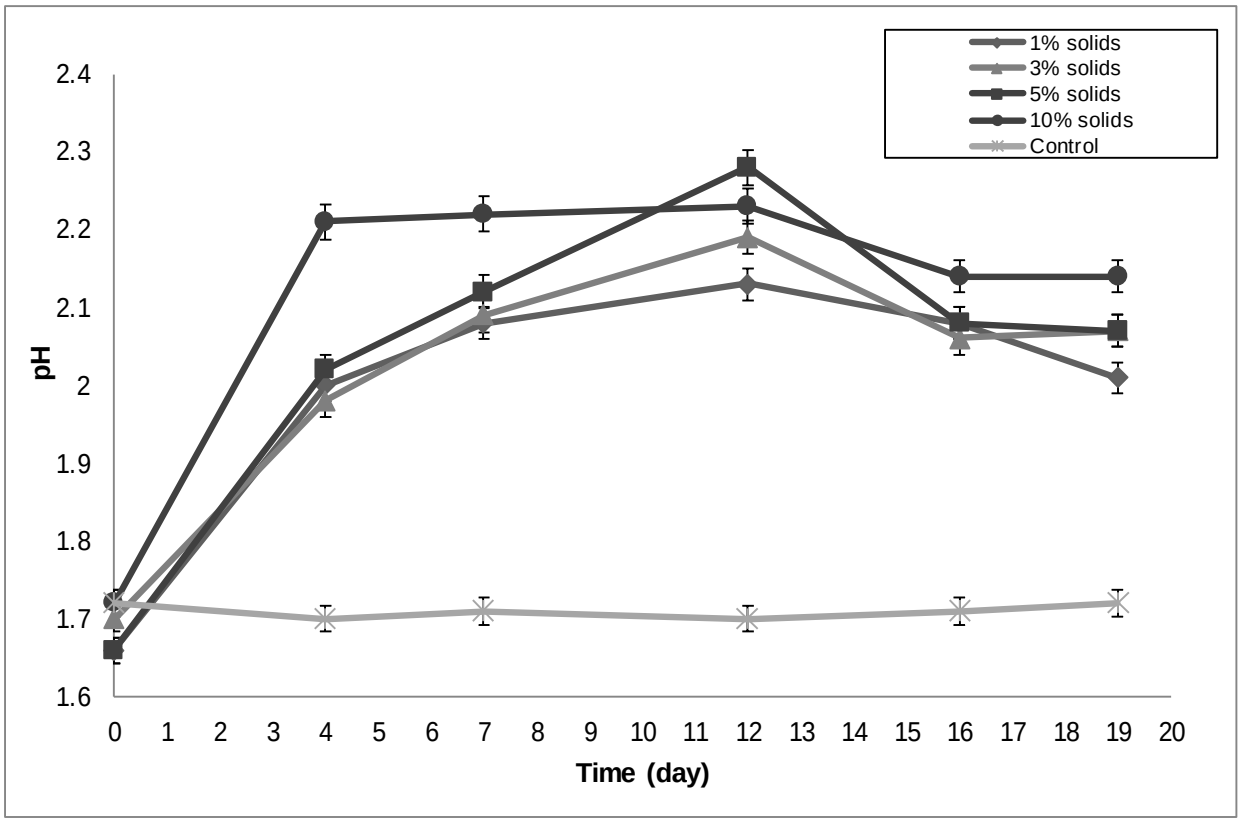

Fig 4. Changes in pH versus solids content in the presence of mutant bacteria

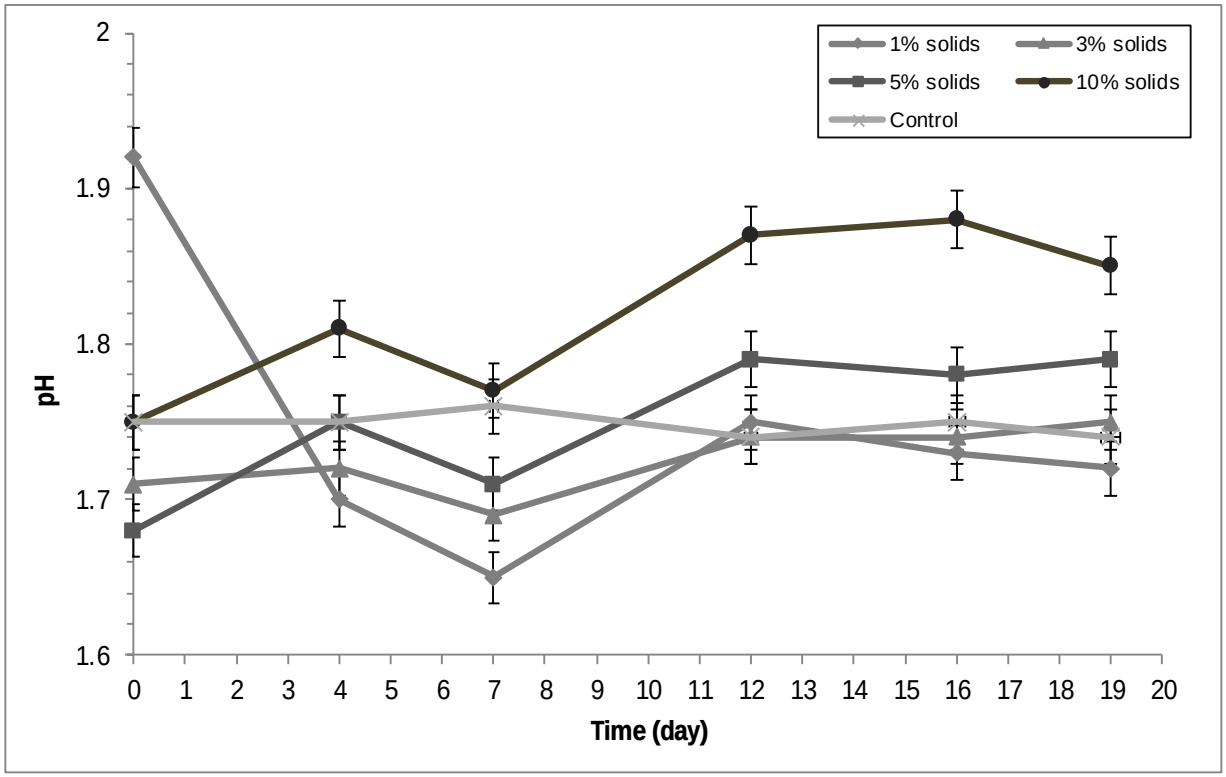

Figure 5. Changes in pH versus solids content in the presence of mutant bacteria in 9K medium with the iron free solution

Changes in $[Fe^{3+}]/[Fe^{2+}]$ ratios and concentrations of Fe^{3+} in the medium are reflected by changes in Eh (Figs. 6-9). Because the oxidation of Fe^{2+} provides energy for bacterial growth, the concentration of Fe^{3+} and Eh in bacterial cultures decreases with time. However, in the present experiments, pH reached a maximum in the culture system and Eh became stable. In further experiments, growth of bacteria was faster in the presence of 10% solids and the best oxidation activities and growth rates were achieved within 12 and 8 days in wild type and UV mutant bacteria, respectively (Figs. 10-13).

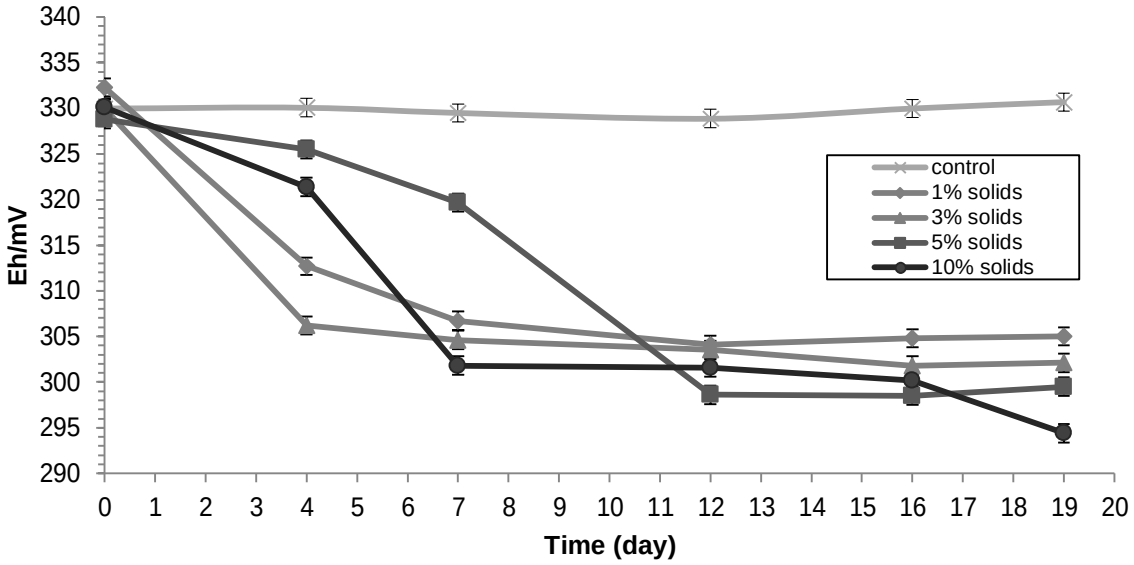

Fig 6. Changes in Eh versus solids content in the presence of wild type bacteria

Fig. 7. Changes in the Eh versus solids content in the presence of wild type bacteria in the iron-free solution

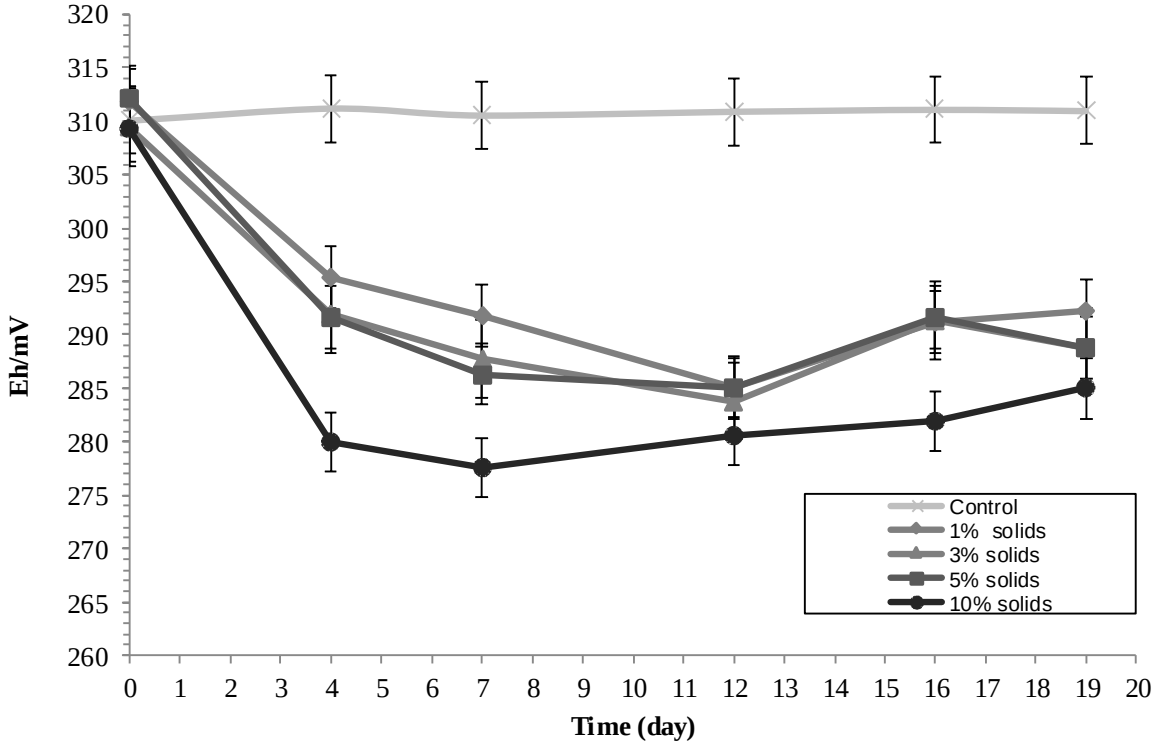

Figure 8. Changes in Eh versus solids content in the presence of mutant bacteria

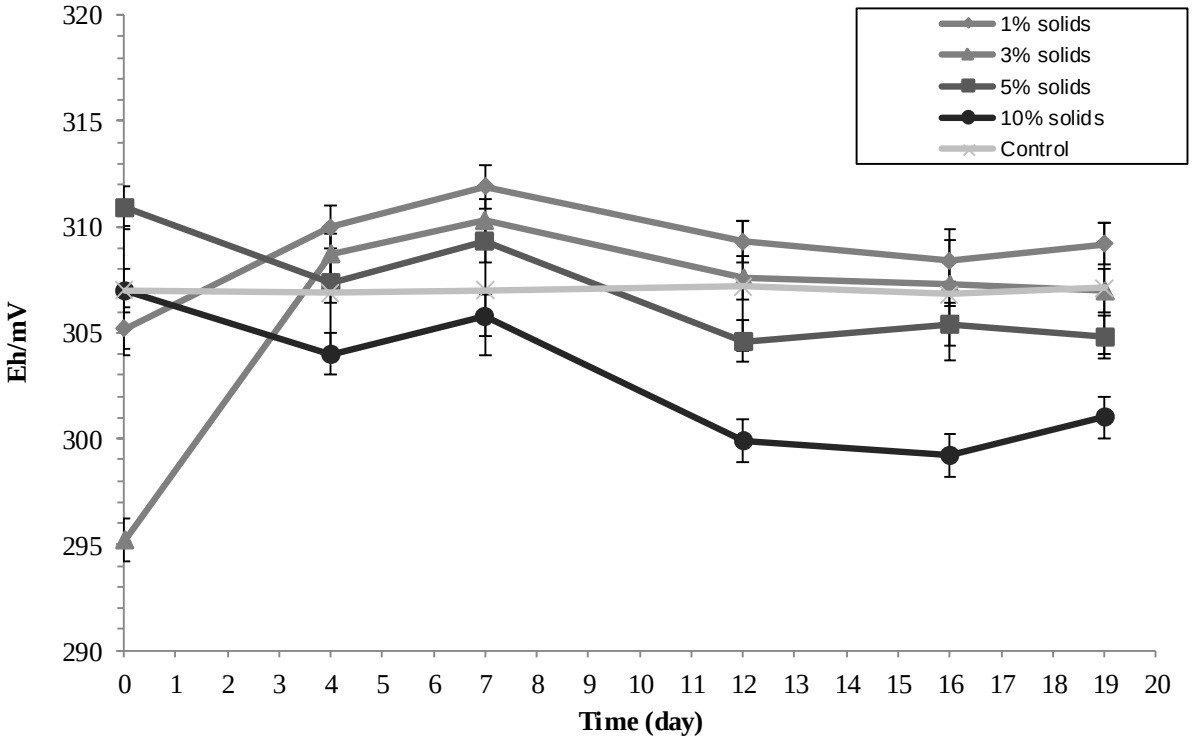

Fig 9. Changes in Eh versus solids content in the presence of mutant bacteria in 9K medium with the iron-free solution

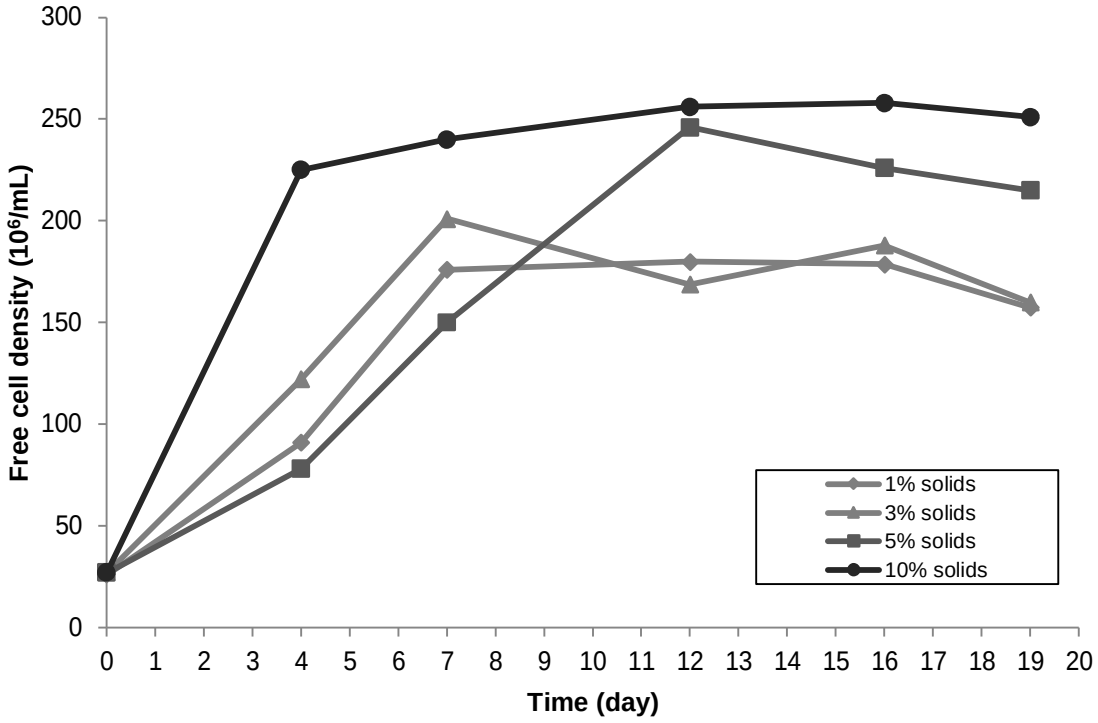

Fig 10. Growth curves of wild type bacteria in the presence of various percentages of solids

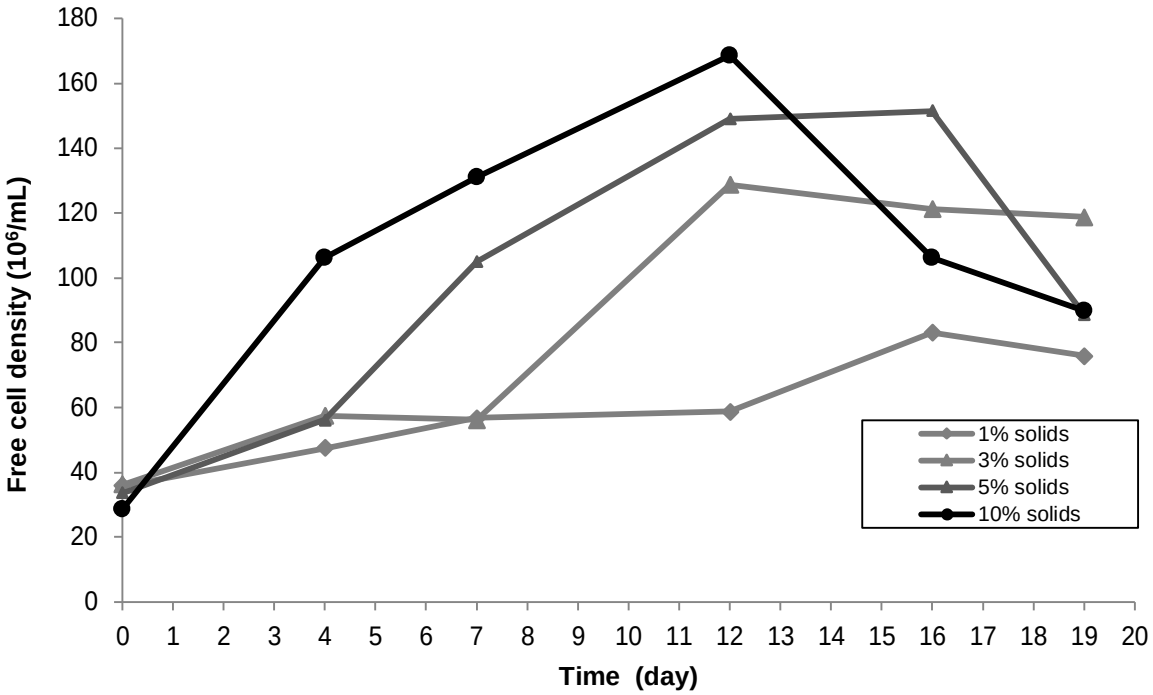

Fig 11. Growth curves of wild type bacteria in the presence of various percentages of solids in 9K medium with the iron-free solution

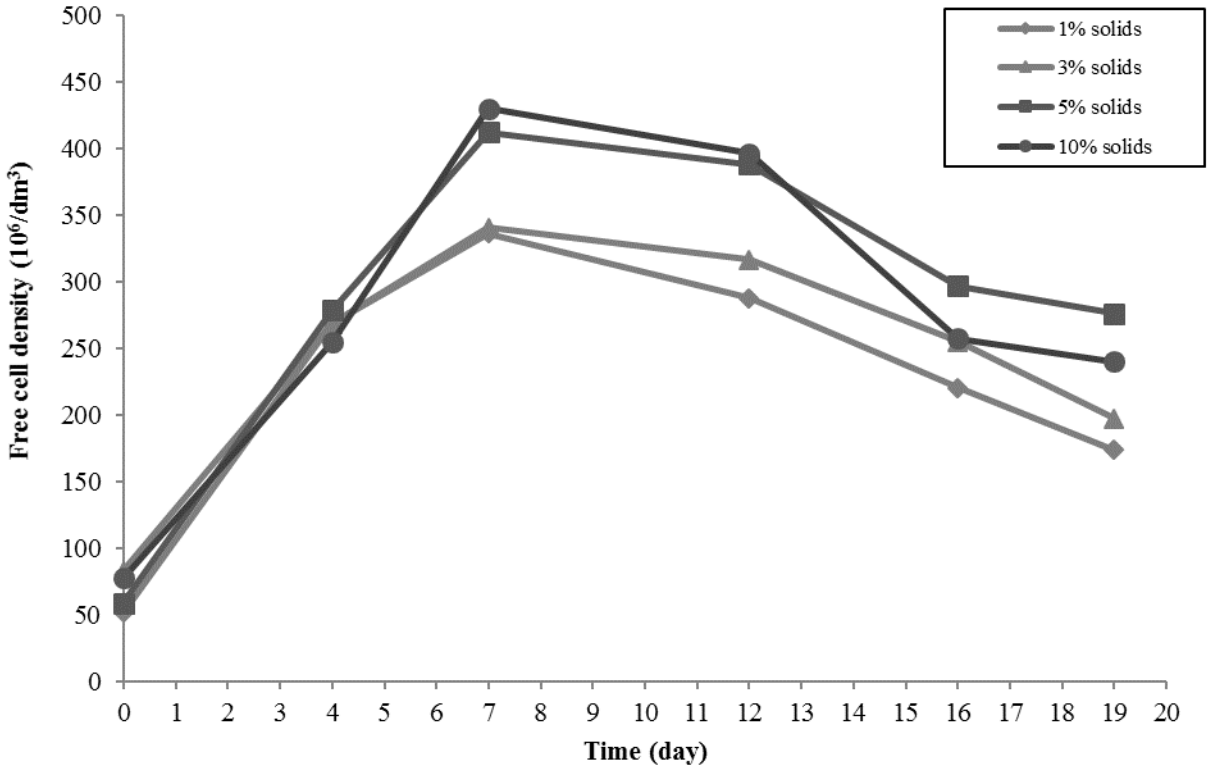

Figure 12. Growth curves of mutant bacteria in the presence of various percentages of solids

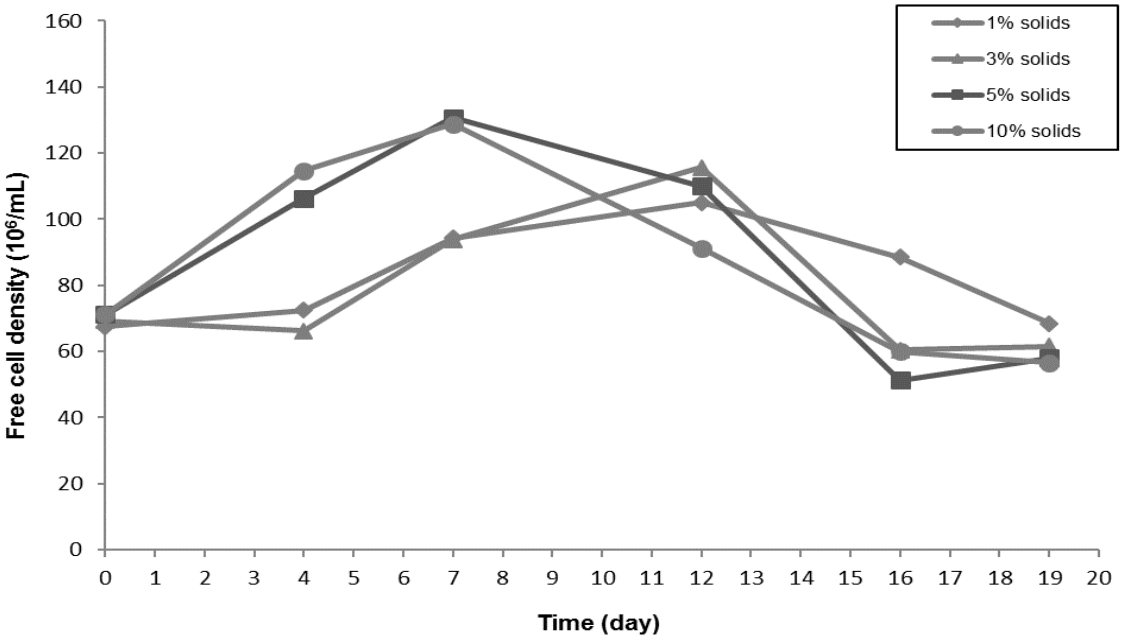

Fig 13. Growth curves of mutant bacteria in the presence of various percentages of solids in 9K medium with the iron free solution

Iron extraction from refractory gold ores was monitored over time in the presence of A. ferrooxidans at various pulp densities (Figs. 14-17). In these experiments, the highest Fe dissolution rate was approximately 55% after 7 days of bio-oxidation in the presence of

1% solids. Moreover, Fe dissolution was 99.17% after 16 days of bio-oxidation with mutant bacteria in the presence of 1% solids (Fig. 15). However, according to XRD patterns, no changes in mineralogical composition of the ore were observed after bio-oxidation (Fig. 18).

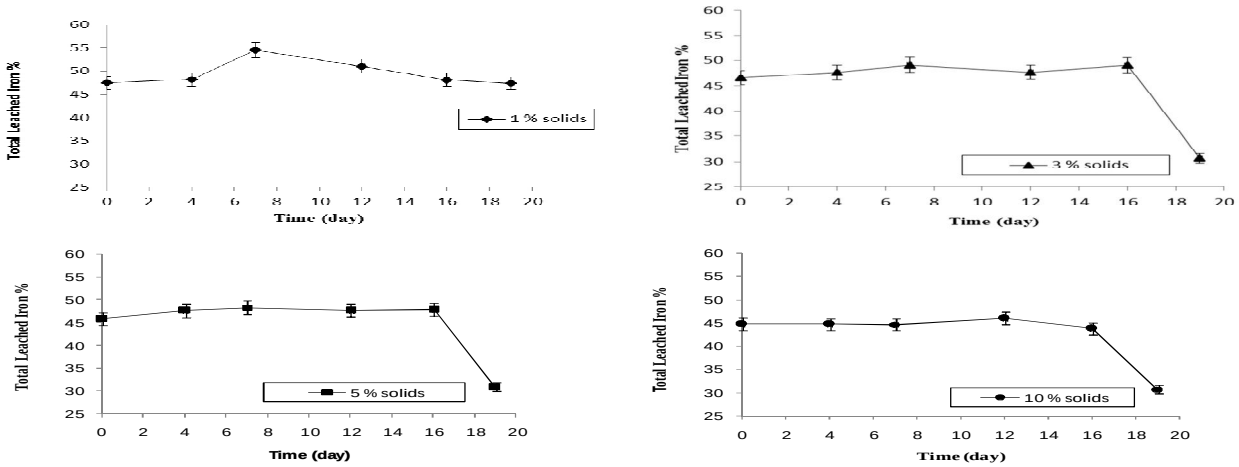

Fig 14. Effect of wild type bacterial pulp density on iron dissolution efficiency

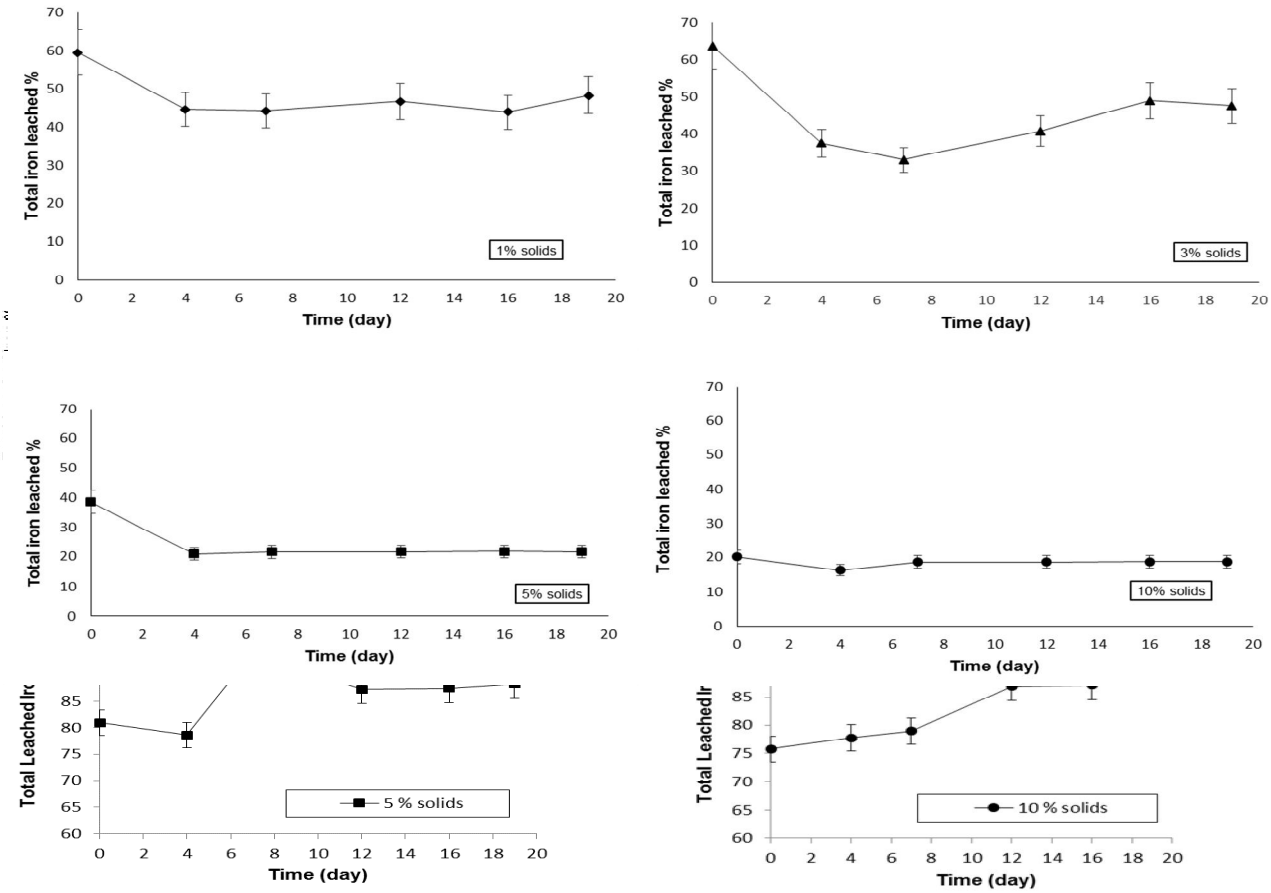

Fig 16. Effect of wild bacterial pulp density on iron dissolution efficiency in 9K medium with the iron free solution

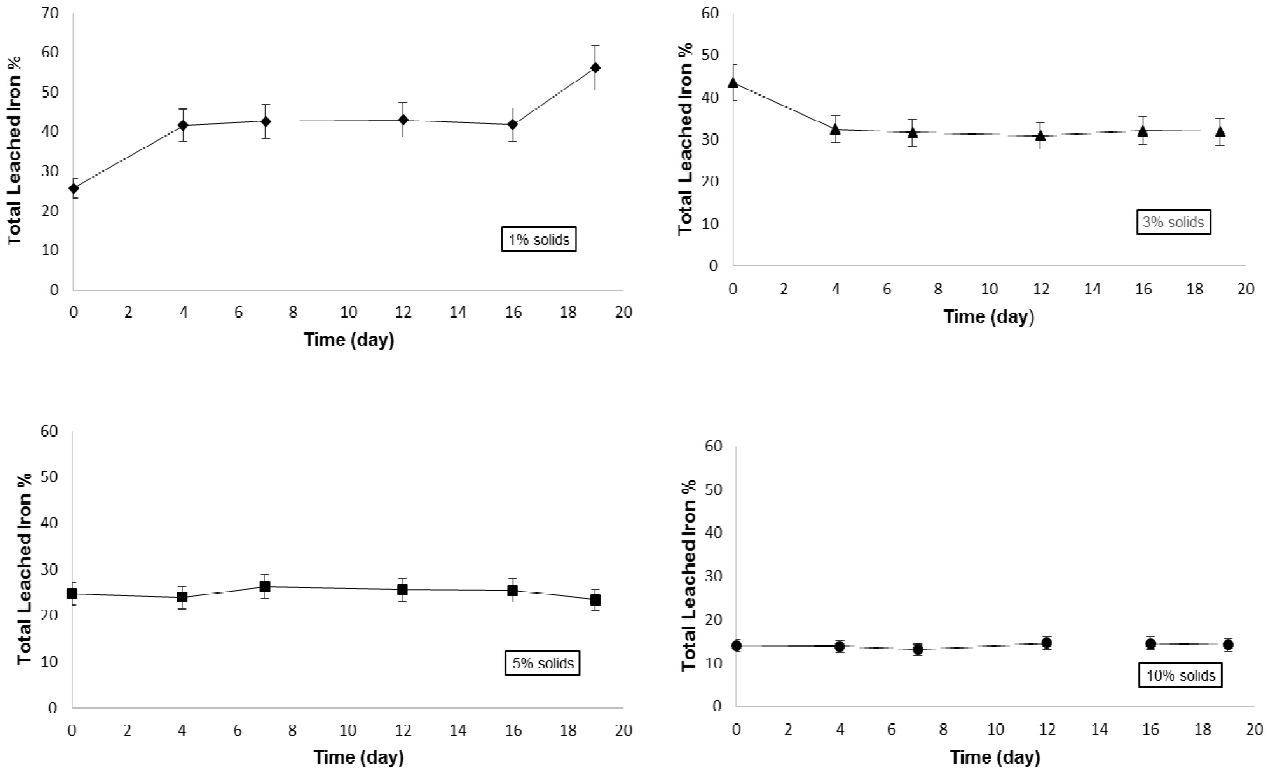

Fig 17. Effect of mutant bacterial pulp density on iron dissolution efficiency in 9K medium with the iron free solution

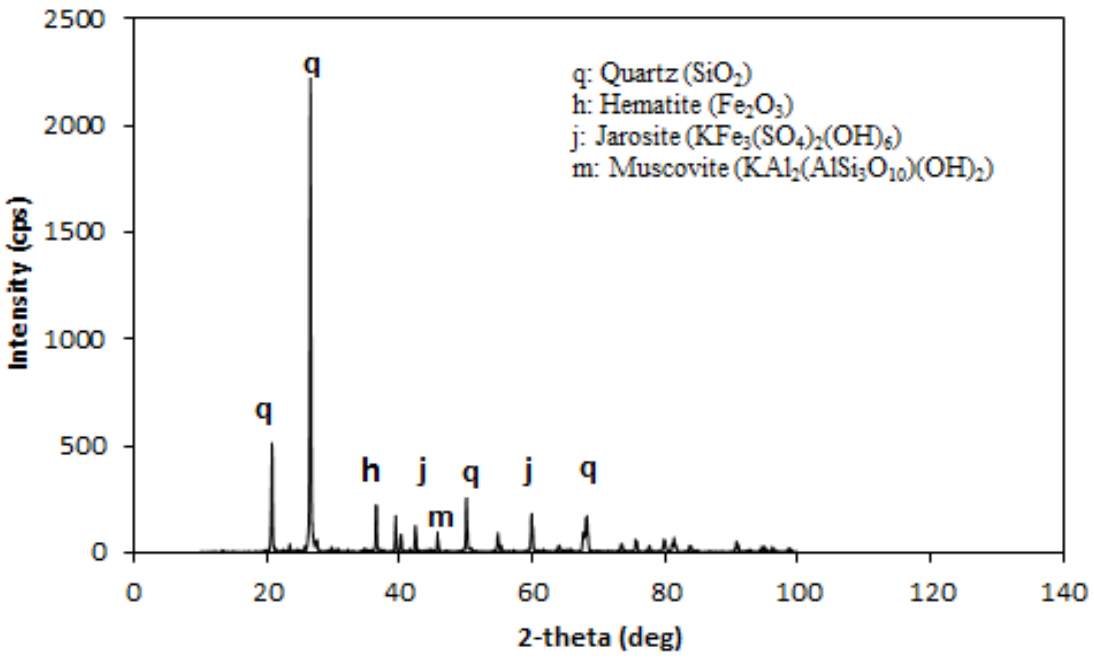

Fig 18. XRD analysis of the precipitate after bio-oxidation

IV. CONCLUSION

In this study, bio-oxidation tests with mesophilic (A. ferrooxidans) bacteria culture were performed using refractory gold ore from the Ovacik Gold Mine, Turkey. Bio-oxidation of the refractory gold ore was achieved using mutant cultures of A. ferrooxidans following 5 min of UV exposure and conventional cyanide leaching processes. The present data indicate a Fe dissolution efficiency of 99.17% using the mutant bacteria in the presence of 1% solids.

It is widely accepted that iron dissolution efficiency decreases with increasing bacterial pulp densities. Moreover, Ciftci and Akcil (2010) showed superior oxidation activities of mixed mesophilic bacterial cultures at high solid ratios. However, increases in solid ratios to >5% w/v resulted in decreased oxidation activities of microorganisms [23].

Previously, iron dissolution efficiencies decreased with time because of accumulating jarosite ($KFe_3(SO_4)_2(OH)_6$) with iron and silver, resulting in increased sulphate contents of oxidised gold ore and potentially reflecting high redox potentials. In a similar study, Prayuenyong (2002) used physical, chemical and biological techniques in a 'Process of Coal Bio-desulphurisation', and recorded the emergence of jarosite as hydronium, potassium, sodium and ammonium precipitates [24]. This is an important problem of pre-oxidation, and Ciftci and Akcil (2010) showed that these components react with and produce cyanide as jarosite components emerge and precipitate.

Bioleaching/biooxidation has been commercially used as a pre-treatment for gold ores with high sulphur and iron contents. In Turkey, there are significant quantities of gold and silver containing refractory ores for which biooxidation can be applied successfully. The idea of using bioleaching/biooxidation as a pre-treatment step can be more effective when using mutant cultures of A. ferrooxidans following 5 min of UV exposure with a combination of conventional cyanide leaching processes.

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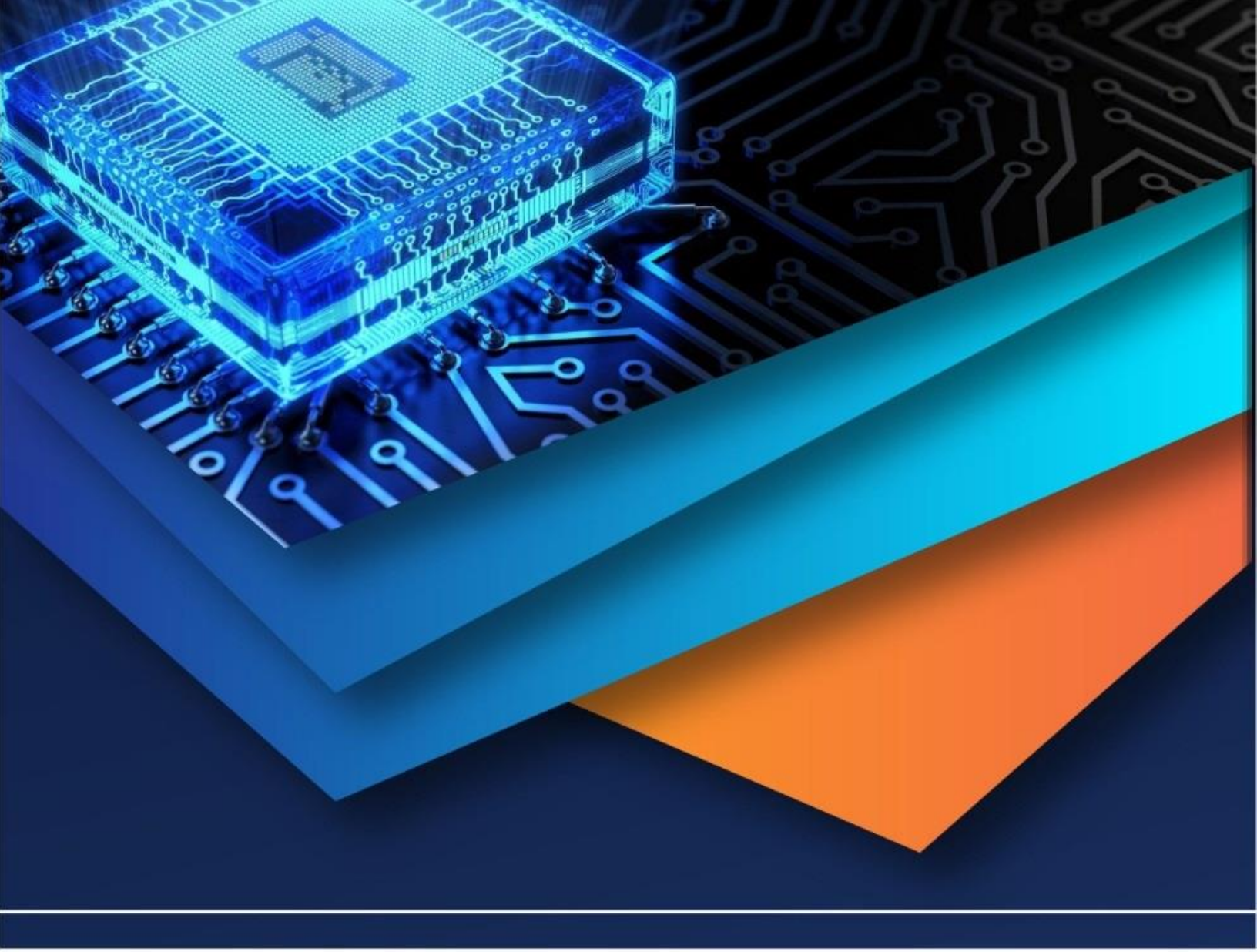

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